UNIVERSIDADE FEDERAL DO PARANÁ

MARCO COLOSSI BRUSTOLIN

RELATIONSHIPS BETWEEN THE SPATIAL VARIATION OF MEIOBENTHIC COMMUNITIES AND LANDSCAPE ATTRIBUTES
RELAÇÃO ENTRE A VARIAÇÃO ESPACIAL DE METACOMUNIDADES MEIOFAUNAIS E ATRIBUTOS DA PAISAGEM

PONTAL DO PARANÁ

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em SISTEMAS COSTEIROS E OCEÂNICOS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MARCO COLOSSI BRUSTOLIN** intitulada: **Relação entre a variação espacial de meta-comunidades meiofaunais e atributos da paisagem**, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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"Quem tem consciência para ter coragem
Quem tem a força de saber que existe
E no centro da própria engrenagem
Inventa a contra-mola que resiste

Quem não vacila mesmo derrotado
Quem já perdido nunca desespera
E envolto em tempestade decepado
Entre os dentes segura a primavera"

João Apolinário

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Cheers!

“Nunca sei ao certo
Se sou menino de dúvidas
ou um homem de fé

certezas o vento leva
só dúvidas continuam de pé”

Paulo Leminski

RESUMO

Ao longo desta tese, investiguei as relações entre nematoides de vida livre e a heterogeneidade da paisagem em sedimentos inconsolidados. Os padrões de variação na estrutura da comunidade foram avaliados em múltiplas escalas espaciais e temporais, usando uma combinação de abordagens experimentais e diversas ferramentas estatísticas, organizadas em quatro capítulos diferentes. Ao fazer isso, tentei inferir alguns dos mecanismos subjacentes à montagem e à dinâmica da metacomunidade de nematoides marinhos, e como a mudança climática pode afetá-los. Os capítulos individuais foram orientados por três pontos principais dentro da ecologia de metacomunidades: 1) a estrutura das metacomunidades em paisagens dinâmicas heterogêneas; 2) o efeito de mudanças de longo prazo na dinâmica da metacomunidade; 3) a importância da variabilidade espaço-temporal em pequena escala para monitorar as metacomunidades.

Palavras-chave: Biodiversidade. Nematoda. Ecologia da Paisagem. Macroecologia. Meiofauna. Distribuição Espacial. Dinâmica Temporal. Mudanças Climáticas. Aquecimento. Acidificação.

ABSTRACT

Throughout this thesis, I investigated the relationships between free-living nematode fauna and soft-sediment landscape heterogeneity. The variation patterns in community structure across multiple spatial and temporal scales were assessed using a combination of several statistical tools and experimental approaches that are organized into four different chapters. By doing this I tried to infer some of the underlying mechanisms driven metacommunity assembly and dynamics, as well how climate change may affect them, laying light over new analytical perspectives and interpretations in nematode ecology. The individual chapters were oriented by three main issues of metacommunity ecology: 1) the structure of metacommunities in dynamic heterogeneous landscapes; 2) the effect of long-term changes in metacommunity dynamics; 3) the importance of small-scale spatiotemporal variability for monitoring metacommunities.

Key-words: Biodiversity. Free-living marine nematodes. Landscape structure. Macroecology. Meiofauna. Spatial distribution. Temporal dynamics. Climate Change. Warming. Acidification

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PREFACE

The marine soft-bottom is one of the biggest ecosystems of our world, extending from intertidal estuarine sandbanks to deep-sea abyssal plains (Zajac 2008). Soft-bottom marine landscapes are primarily shaped by physical and geomorphological processes. Horizontal/advection and vertical/diffusion water movements introduce variability in the seafloor topography and sediment composition (e.g., grain size, sorting) over multiple spatial scales (Zajac 2008, Chapman et al. 2010). In addition, bioengineering modify sediment matrix, increasing heterogeneity and patchiness (Meysman et al. 2006, Kristensen et al. 2012). Also, life history traits, predation and competition, as well other biological interactions acting at small-scales can affect metacommunity assembly and dynamics over larger spatiotemporal scales (Chave 2013, Richardson et al. 2014).This interdependence between processes and scales lead to the recognition that defining the scale at which ecological processes operate is pivotal to produce general predictions (Cottenie 2005, Chave 2013).

This multiscale perspective over community ecology brought a more holistic view on how species interact with each other and with the environment. In this sense, the concept of metacommunities, that are composed by a set of smaller local communities linked by dispersal established a foundation for new and exciting theorizations about the dynamic of ecological interactions (Leibold et al. 2004b, Holyoak et al. 2005)

In soft-sediment landscapes, small-scale variability sometimes can be higher than large-scale spatiotemporal patterns (Morrisey et al. 1992, Chapman et al. 2010). Thus, quantify this variability and investigate their causes remains a fundamental issue in soft-bottom ecology. In shallow soft-bottoms, transitional gradients between terrestrial and marine ecosystems creates a complex and dynamic environment where habitats boundaries are difficult to recognize (Thrush et al. 2013, Valanko et al. 2015). These difficulties led to a belated development of empirical and theoretical studies on metacommunity structure and dynamics at the marine soft-sediment habitats (Zajac 2008, Valanko et al. 2015). Hence, we still trying to understand how metacommunities are organized and which are the underlying mechanisms structuring them.

Despite this, the impacts of human disturbances and climate changes on biodiversity are already being observed (Zeppilli et al. 2015, Nagelkerken and

Connell 2015). Recent predictions account for complex impacts of ocean acidification and warming on food-web productivity and trophic interactions (Goldenberg et al. 2017). Also, run-offs of muddy sediments from drainage basins into coastal environments will affect ecosystem function and bioengineering (Pratt et al. 2014). However, the effects of climate change on metacommunity assembly and dynamics remain unclear.

Throughout this thesis, I investigated the relationships between free-living nematode fauna and soft-sediment landscape heterogeneity. The variation patterns in community structure across multiple spatial and temporal scales were assessed using a combination of several statistical tools and experimental approaches that are organized into four different chapters. By doing this I tried to infer some of the underlying mechanisms driven metacommunity assembly and dynamics, laying light over new analytical perspectives and interpretations in nematode ecology.

The individual chapters, oriented by three main issues of metacommunity ecology:

1) the structure of metacommunities in dynamic heterogeneous landscapes
2) the effect of long-term changes in metacommunity dynamics
3) the importance of small-scale spatiotemporal variability for monitoring metacommunities.

The first chapter is a meta-analysis summarizing the macroecological patterns of spatial variation on nematode richness in tropical and subtropical mangrove sediments. The study investigates the relationship of local and regional nematode richness with mangrove landscape attributes (e.g., forest size and complexity, diversity of mangrove tree species, and aboveground biomass production), as well with climate drivers (i.e., temperature) and biogeographical boundaries (Renema et al. 2008, Stöhr et al. 2012, Leprieur et al. 2016).

The second chapter is a regional scale study, assessing the spatial variation of nematode communities across three distinct habitats (mangrove sediments, estuarine tidal flats and sandy beaches) over multiples spatial scales (10 m to 150 km). This chapter explores the relationships between variation within and among soft-bottom coastal habitats. Variance partitioning (Cottenie 2005, Brown et al. 2017) and *elements of metacommunity structure* approaches (EMS, Leibold & Mikkelson 2002, Presley et al. 2010) were used to assess the influence of gradient/transition

zones in metacommunity organization. The potential roles of environmental, spatial, and ecological drivers, as well the underlying mechanisms controlling community assembly and dynamics are further discussed in details, i.e., patch-dynamics, species-sorting, mass-effect and neural (Leibold et al. 2004b, Hubbell 2005).

The third chapter deals with the temporal variations on community structure at local and landscape scales (i.e., nearby habitats sharing the same species pool and regional context). Variation in community structure was investigated over multiple temporal scales (days, weeks, months, and seasons) in two subtropical coastal habitats characterized by different hydrodynamic and physicochemical regimes (a protected estuarine tidal flat versus an exposed sandy beach). The environmental drivers of community temporal patterns at each habitat, as well the implications of these differences in metacommunity organization are explored in more details throughout this chapter.

The fourth chapter deals with responses of benthic metacommunities to warming and ocean acidification. A large mesocosm experiment was set-up to investigate how future scenarios of ocean acidification and warming affect metacommunity organization and dynamics along three coastal habitats: bare sands, artificial seagrass, and rocky shores. The causes and consequences of these shifts in metacommunity organization and dynamics are discussed more profoundly along this chapter.

Finally, in the general discussion, I bring an overview of most relevant findings, as well the relationships among the individual chapters.

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1 CHAPTER 1: LARGE-SCALE DISTRIBUTION PATTERNS OF MANGROVE NEMATODES – A GLOBAL META-ANALYSIS

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Authors contribution statement

Neither the manuscript nor any significant part of it is under consideration for publication elsewhere, nor has it appeared elsewhere in a manner that could be construed as a prior or duplicate publication of the same, or very similar, work. All of the undersigned authors participated actively in the study, and none has any potential conflict of interest. All of the authors have taken part in data analyses and interpretation. All of them have read and approved the manuscript in its present form and have agreed to its submission to Ecology and Evolution.

MCB, IN and GF, conceived the ideas; MCB compiled and analyzed the data with additional help of GF and IN; MCB led the writing with assistance from IN and GF.

Abstract

Aim: Mangroves harbor diverse invertebrate communities, suggesting that macroecological distribution patterns of habitat-forming foundation species drive the associated faunal distribution. Whether these are driven by mangrove biogeography is still ambiguous. For small-bodied taxa, local factors and landscape metrics might be as important as macroecology. We performed a meta-analysis to address the following questions: (1) can richness of mangrove trees explain macroecological patterns of nematode richness?; and (2) do local landscape attributes have equal or higher importance than biogeography in structuring nematode richness?

Location: Mangrove areas of Caribbean-Southwest Atlantic, Western Indian, Central-Indo-Pacific and Southwest Pacific biogeographic regions.

Methods: We used random-effects meta-analyses based on natural logarithm of the response ratio (lnRR) to assess the importance of macroecology (i.e., biogeographical regions, latitude, longitude), local factors (i.e., above-ground mangrove biomass and tree richness), and landscape metrics (forest area and shape) in structuring nematode richness from 34 mangroves sites around the world.

Results: Latitude, mangrove forest area and forest shape index explained 19% of the heterogeneity across studies. Richness was higher at low latitudes, closer to the

equator. At local scales, richness increased slightly with landscape complexity and decreased with forest shape index.

Main conclusions: Our results contrast with biogeographic diversity patterns of mangrove-associated taxa. Global-scale nematode diversity may have evolved independently of mangrove tree richness, and diversity of small-bodied metazoans is probably more closely driven by latitude and associated climates, rather than local, landscape or global biogeographic patterns.

Keywords: Biodiversity, Free-living marine nematodes, Landscape structure, Macroecology, Meiofauna, Spatial distribution

1.1 INTRODUCTION

The hotspot of tropical marine biodiversity observed in the Indo-West Pacific (IWP) region is a well-recognized macroecological pattern described for many coastal and marine plant and animal species, although neither the processes nor the mechanisms responsible for this are well understood (Bowen et al. 2013). Studies on mangroves and associated macroinvertebrate species corroborate this pattern, as well as studies on coral reefs and their associated fish and foraminifera communities (Ellison 2008; Renema et al. 2008; Bellwood and Meyer 2009; Gaither and Rocha 2013). The epicenter of diversity in the IWP has been traditionally associated with Pleistocene sea level changes and the geographical complexity of the area (Bellwood et al. 2005), but molecular and fossil evidence from a range of taxa contradicts this notion and points to the presence of lineages from the Miocene, being much older than previously thought (Renema et al. 2008). Alternative hypotheses for its high biodiversity are that the IWP region may act either as a center of origin, overlap or accumulation (Bellwood and Meyer 2009; Bowen et al. 2013). Biodiversity hotspots have moved across almost half the globe over the past 50 million years with the timing and locations of their epicenter occurrences coinciding with major tectonic events (Renema et al. 2008, Leprieur et al. 2016). In this case, biodiversity hotspots are a product of ecological processes operating over geological time scales.

In relation to mangroves, one hypothesis is that they evolved around the

Tethys Sea during the Late Cretaceous, and regional species diversity resulted from *in situ* diversification after continental drift (Ellison et al. 1999). Mangrove tree species are uniquely adapted to tropical and subtropical coasts, and although they have a relatively low number of species, mangrove forests provide at least US $1.6 billion each year in ecosystem services supporting coastal livelihoods worldwide (Polidoro et al. 2010). Globally, mangrove forests are declining rapidly as they are cleared for coastal development, aquaculture and logged for timber and fuel production (Ellison 2008; Hutchison et al. 2014). This extensive habitat loss and fragmentation is generating extinctions and shifts in biodiversity with impacts on ecosystem functions and services (Thrush et al. 2008; Snelgrove et al. 2014).

Little is known about the effects of mangrove forest area on local and regional populations of mangrove species and its associated fauna and flora (Nagelkerken et al. 2008; Polidoro et al. 2010). The aerial roots of mangroves partly stabilize the environment and provide a substratum on which many species of plants and animals live, while their leaf litter is transformed into detritus through herbivory, supporting complexes food webs (Somerfield et al. 1998; Nagelkerken et al. 2008). The presence of mangrove pneumatophores increases algal retention, and therefore the density and diversity of associated meio- and macroinvertebrates (Gwyther and Fairweather 2005; Bishop et al. 2012). Hence, ecosystem engineering, facilitation cascades, and niche construction may have had an important role in generating and maintaining biodiversity of associated fauna at evolutionary time-scales (Erwin 2008). However, mangrove deforestation may result in biodiversity losses (Ellison 2008; Polidoro et al. 2010) and change the biomass size spectrum of meiofaunal communities, e.g. favoring small-bodied non-selective deposit feeders above less generalist functional groups and species (Sabeel and Vanreusel 2015). Generally, nematodes are the most abundant and diverse meiofaunal group inhabiting marine sediments. They play an important role in the remineralization of organic matter, and because they feed on a wide range of food items and have high functional diversity, they act as a critical link between microorganisms and higher food-web levels (Hamels et al. 2001; Pinto et al. 2013). Mangrove interstitial fauna is tightly associated to sedimentary micro-niches (Alongi 1987; Pinto et al. 2013), and the higher richness in the IWP may be partly caused by variations in regional geomorphological complexity and habitat heterogeneity among ecoregions. Despite this, studies on the distribution of mangrove benthic fauna are mostly restricted to

local scale patterns (Mokievsky et al. 2011). On a global scale, it could be hypothesized that the longitudinal and latitudinal gradients in mangrove tree richness will drive richness of the associated fauna. Particularly for estuarine nematodes, it has been suggested that global patterns are better explained by the moderate endemicity hypothesis (MEH), which suggests that nematodes are dispersion-limited and their distribution is influenced by local and regional environmental conditions rather than a homogeneous distribution across the globe (Fontaneto 2011; Fonseca and Netto 2015).

Estimates of the number of meiofauna species inhabiting mangrove sediments vary widely and come from a heterogeneous set of mangrove habitat types (Nagelkerken et al. 2008). Mangroves can differ in their size and shape, and mangroves with a different perimeter-to-area ratio might affect the structure of associated fauna differently (Boström et al. 2011). Furthermore, mangroves with a similar total surface area and shape can differ in their above-ground biomass per unit of area, as well as their flow through adjacent habitats (Boström et al. 2011). However, whether such landscape heterogeneity explains heterogeneity in nematode richness within mangrove forests is still unknown.

We here address the following questions: (1) can species richness of mangrove trees from different ecoregions (*i.e.*, Central Indo-Pacific, Southwest Pacific, Western Indian and Caribbean/Southwest Atlantic regions) explain large-scale spatial patterns of nematode richness?, and (2) do local landscape attributes have equal or more importance than biogeographic patterns in structuring nematode richness? To evaluate which factor is more important in determining nematode richness, a random-effects meta-analyses of published studies from around the world was performed.

1.2 METHODS

1.2.1 Data Selection

The literature search was based on studies on nematode fauna from mangrove forests, published in journals indexed in Thomson Reuters' Web of Science, using the search strings "nematodes" + "mangrove". We restricted our literature review to peer-reviewed papers written in English. From the ~5,950

published articles, 25 studies (covering 34 study sites) were included in the analyses (Fig.1). A list of the data sources is found in Appendix 1. Mangrove forests within a study that were located in different estuaries were considered as different analytical units (sites). Only studies that reported average values accompanied by some measure of variance of nematode species richness were selected (Supporting information Table S1). The average nematode richness per study was based on the arithmetic mean of samples from different sites. Sieve size and core volume were obtained from the same literature to evaluate the effects of sampling artifacts on nematode richness. The latitudinal distribution of mangrove tree richness was based on Ellison et al. (1999) and Ellison (2008). Ellison's data sets are composed of a presence/absence list of mangrove tree species across geographical coordinates. Mangrove tree richness was estimated as the total number of tree species occurring at the same degree of latitude and longitude for which diversity data of nematodes were available. Aboveground biomass, as well as spatial attributes of mangrove forests, like cover area and shape index, were obtained from modelled data sets (Giri et al. 2011; Hutchison et al. 2014). Above-ground mangrove biomass, mangrove forest cover area, and forest shape index of each mangrove forest included in the analyses were extracted from the original shapefiles through geostatistical tools available in the Quantum GIS software. Shape index was calculated as Perimeter / (2 * SquareRoot (PI * Area)) and measured using the Polygon Shape Indices module in SAGA-GIS (Lang and Blaschke 2007).

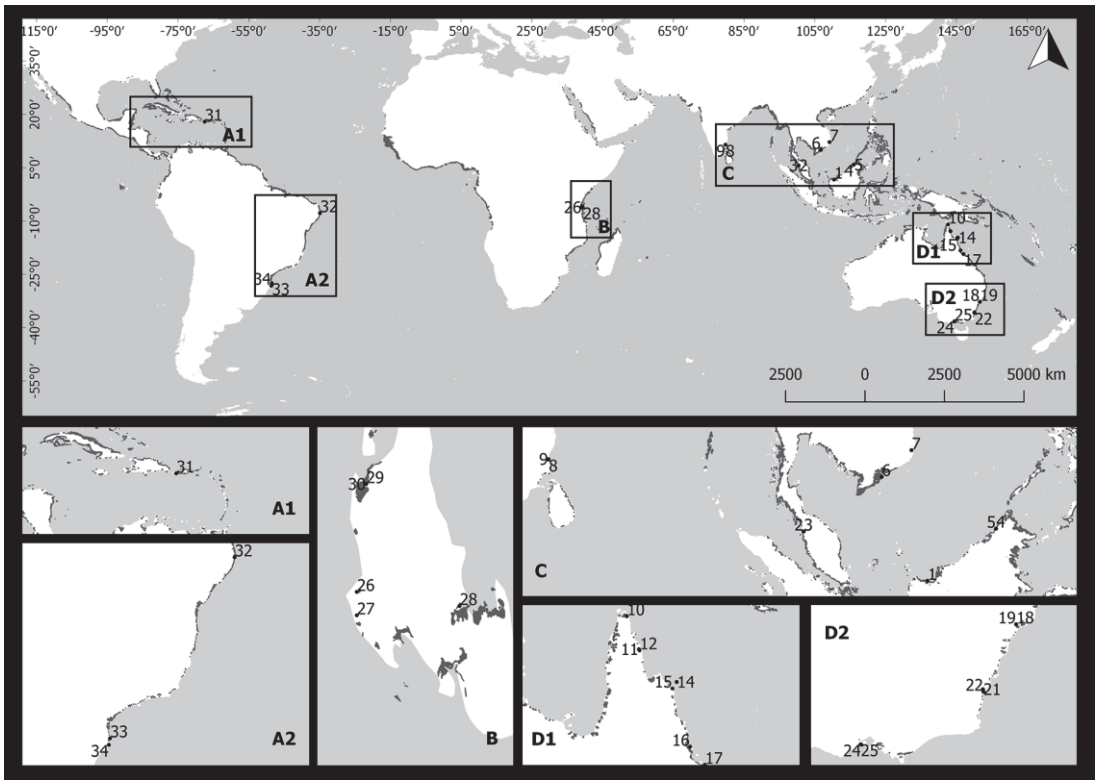

Fig. 1 Global distribution of studies (n = 34) on mangrove nematode richness up to year 2016 in each Marine Biogeographic Region: Caribbean-Southwest Atlantic (A1 and A2); Western Indian (B); Central-Indo-Pacific (C); Southwest Pacific (D1 and D2)

1.2.2 Meta-analyses

In a meta-analysis, results of independent studies are expressed as an index of effect. These effect size estimates are then combined across studies generating a summary of the outcomes. Also, subsets of studies can be examined separately to determine whether their outcomes differ or not (Hedges et al. 1999). For each study, effect sizes of nematode species richness were calculated using the natural logarithm of the response ratio (lnRR). The log response ratio (lnRR) was used as an effect size because of its robustness to natural variability of ecological data and small sample sizes (Lajeunesse and Forbes 2003). Traditionally, the effect size based on lnRR represents the ratio of the response variable measured in an experimental group to that of the control group (Hedges et al. 1999). In our study, the most distant site from the IWP hot-spot (i.e., higher latitudes in the Atlantic Ocean) was used as the reference site, and species diversity of all other studies were compared relative to this site. In such way, we evaluated the magnitude of change in diversity along an east/west spatial gradient.

Analyses were carried out using the R (version 3.3.1; R Development Core Team 2016) package “Metafor” (Viechtbauer 2010). Weighted random-effects models were carried out to calculate a summary effect size. Random-effects analysis assumes that the true effect size differs between experiments, and the estimated summary effect is the mean of the effects observed across the studies. This meant that even if studies had a low weighting, the individual effect sizes from all studies could be incorporated into the summary effect (Borenstein et al. 2009). Both the within-study variance (inverse of the effect size variance) and the between-study variance (σ^2 pooled) were used to weight the studies. Therefore, studies with higher replication and/or lower variance were considered more precise and weighted accordingly (Gurevitch and Hedges 1999; Hedges and Olkin 1985). Between-study variance was estimated using the DerSimonian-Laird method (DerSimonian and Laird 1986). Statistical significance was attributed to each summary effect size by calculating a bias-corrected 95% confidence interval (CI; Hedges and Olkin 1985). If the confidence intervals do not overlap zero, then the effect size is considered significant ($p < 0.05$).

The total heterogeneity of a weighted mean effect size is represented by the Q_T statistic, which is a weighted sum of squares, comparable to the total sum of squares in an ANOVA. For each mean effect size, Q_T was calculated and tested against a χ^2 distribution. A significant Q_T indicates that the variance among individual effect sizes is larger than expected by sampling error and that there may be an underlying structure to the data, and therefore other explanatory variables should be tested (Borenstein et al. 2009). Biogeographic regions and sieve size were treated as categorical factors, while core volume, latitude, longitude, mangrove tree species richness, aboveground mangrove biomass, mangrove forest area, and forest shape index were treated as continuous variables. To evaluate which of those nine explanatory variables were more important for the observed underlying structure on nematode richness a random-effects meta-regression, which is analogous to a multiple linear regression, was performed. For this model, total heterogeneity Q_T can be partitioned in the variance explained by the model (Q_M) and the residual error variance not explained by the model (Q_E). Q_M was tested against a χ^2 distribution and in this case, a significant Q_M indicates statistical differences in the relationship between effects sizes and predictor variables. Between-study variance of lnRR was estimated using the restricted maximum likelihood (REML) method (Viechtbauer

2010). The most parsimonious random/mixed-effect model was chosen based on a step backward selection using Akaike Information Criteria (AIC). In addition, univariate random-effect meta-analyses exploring the individual relationship between the lnRR of nematode richness and all the nine explanatory variables are available in the Supporting information (Table S2).

1.2.3 Sensitivity Analysis

Publication bias and between-study heterogeneity for main effects were tested using Egger's regression test for funnel plot asymmetry (Egger et al. 1997; Sterne and Egger 2005). When a significant relationship between the observed outcomes (i.e., lnRR of nematode richness) and the standard error is detected, then this usually implies asymmetry in the funnel plot, which in turn may be an indication of publication bias. In the absence of bias and between study heterogeneity, the scatter plot will be due to sampling variation alone and the plot will resemble a symmetrical inverted funnel. If the heterogeneity fits with the model's assumptions, then the funnel plot will be symmetrical but with additional horizontal scatter. On the contrary, if heterogeneity is large enough to overwhelm the sampling error then plots become cylindrical (Sterne et al. 2011). The output results from these analyses as well as funnel plots are available in supporting information Table S2 and Fig. S2.

1.3 RESULTS

1.3.1 Large-scale distribution pattern

There was significant heterogeneity (Q_T) across studies ($p < 0.001$, Table S2). Therefore, the importance of several single explanatory variables was tested. At the biogeographic level, positive mean effect-sizes in nematode richness were observed for the Central Indo-Pacific, Southwest Pacific, and Caribbean-Southwest Atlantic (Fig. 2). Biogeographic regions explained alone 24.4% of the total heterogeneity across studies, however, the omnibus test for moderators indicated there were no differences among biogeographic regions ($p = 0.069$, Table S2).

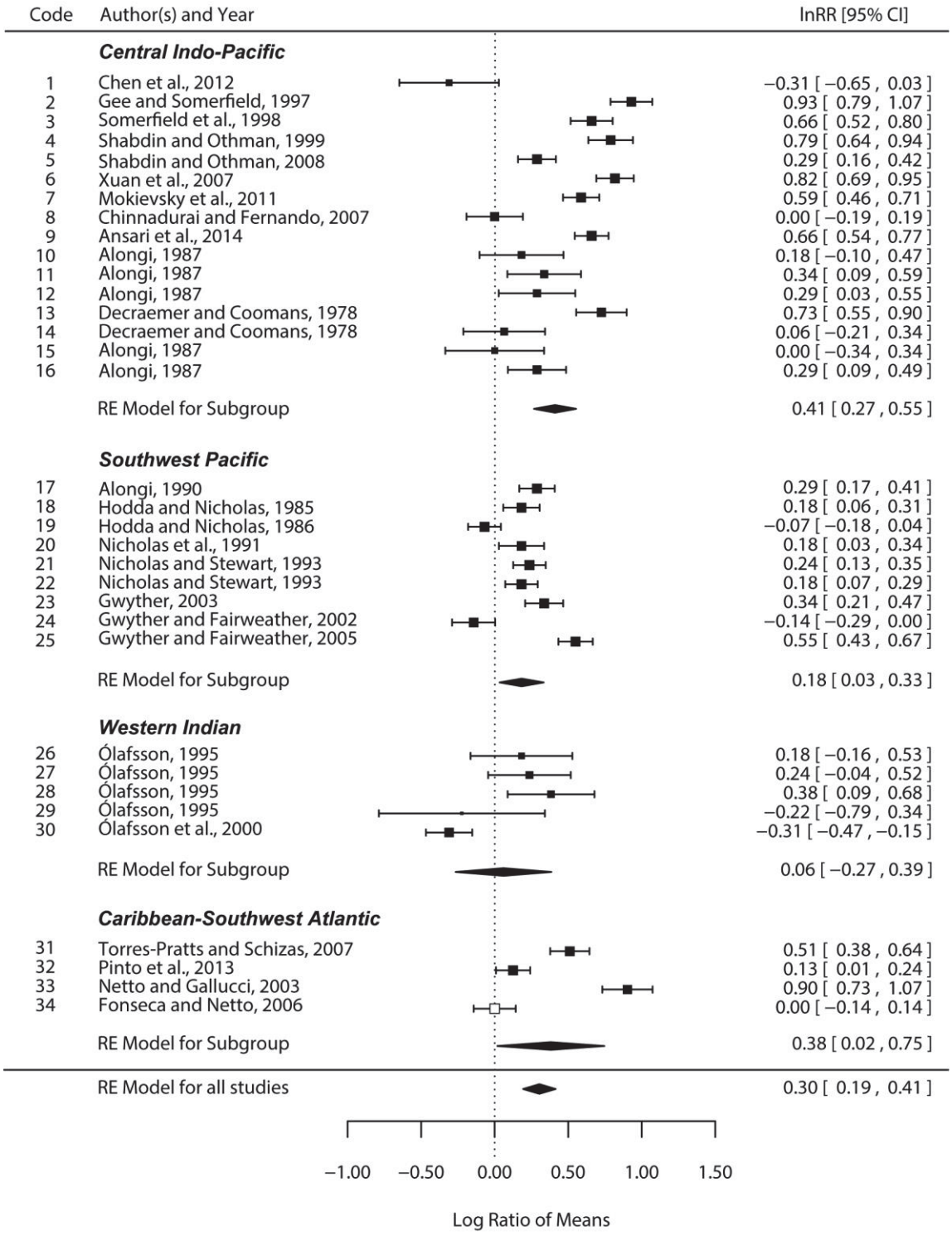

Fig. 2 Natural logarithm of the response ratio (lnRR) of nematode richness. Horizontal black bars are 95% confidence intervals for effect sizes of each study included in the model, the size of the black squares represent the relative weight that each study had on the overall analysis. Open square indicates the farthest study along the east-west gradient, used as reference for the effect sizes estimation. Numbers in right column are average lnRRs with their respective lower and upper confidence intervals. Black diamonds are mean effect sizes for each ecoregion and their length represents confidence intervals

Among all the remaining descriptors, lnRR of nematode richness was only significantly correlated with latitude (Table S2, R^2 = 12.2 %, p = 0.047), with higher richness occurring closer to the equator (Fig. 3). In addition, the multiple meta-regression model revealed latitude, but not biogeographic region as an important macroecological driver of the nematode richness (Table 1).

Tab. 1 Models, number of parameters and values of adjusted Akaike information criteria (AICc), and difference between the model *i* and the best model (ΔAICc), for the alternative models (ΔAICc ≤ 2) explaining Log Response Ratio outcomes from nematode richness of the summarized studies

Model	Parameters	R^2	AIC_c	ΔAIC_c
lnrr ~lat + area + shape + biomass + richness	5	26.95	25.211	1.83
lnrr ~lat + area + shape + biomass	4	25.72	23.707	0.33
lnrr ~lat + area + shape	**3**	**19.35**	**23.377**	**0**
lnrr ~lat + shape	2	14.31	24.622	1.24
lnrr ~lat	1	12.22	24.050	0.67

Log Response Ratio outcomes (lnrr), absolute latitude (lat), total area of mangrove forest (area), shape index of mangrove (shape), total above ground biomass of mangrove (biomass) and number of mangrove tree species at each station (richness).

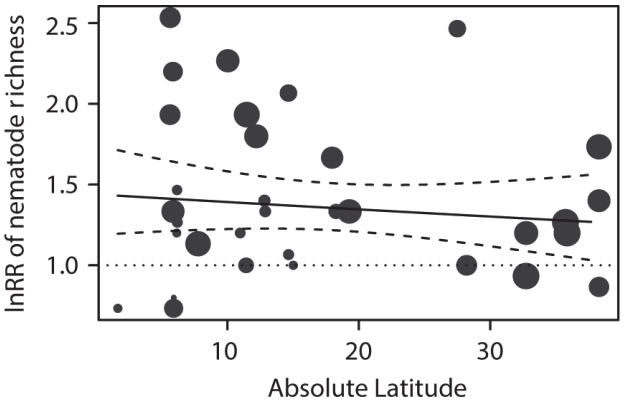

Fig. 3 Scatterplot of the lnRR of nematode richness of the individual studies plotted against absolute latitude. The sizes of the dots are proportional to the inverse of the standard errors (i.e., studies with low internal variability are shown as larger dots). Solid line represents predicted values for a weighted regression line based on a mixed/random-effects model (with corresponding 95% confidence intervals)

1.3.2 Local landscape effects

The most parsimonious multiple meta-regression model according to backward step AIC selection included absolute latitude, total mangrove forest area and mangrove forest shape index as important variables, explaining 19.35 % of the heterogeneity across studies (Table 2). Nevertheless, the two landscape attributes were marginally significant, and were only responsible for 7.1% of total variance explained (see supporting information, Fig. S1). Above-ground tree biomass and richness of tree species had lower importance and did not explain a significant

amount of the heterogeneity in nematode richness as verified by the higher AIC values observed when these variables were included in the multiple meta-regression (Table 1). The Egger's regression test of the fitted model against its standard error indicated that there was a significant asymmetry in the funnel plot (t = -2.062, *df* = 29, *p* = 0.048) which may be an indication of significant between-study heterogeneity. Nevertheless, the funnel plot was symmetrical and horizontally dispersed, which indicates that the heterogeneity fits with the model's assumptions (Fig. S2k).

Tab. 2 Summary of Meta-regression model with the respective values of correlation coefficients, standard errors (SE), t-statistics, lower and upper confidence intervals for each selected explanatory variable. Asterisks represent significance of *p*-values. Amount of variability across studies (I^2) and amount of variability across studies explained by the model (R^2) are stated as percentages. Degrees of freedom (*df1* and *df2*), F-statistic and *p*-value are from the omnibus test of moderators included in the model

	I^2	**R^2**	***df*1**	***df*2**	**F**	***p*-value**
	92.57	19.35	3	30	2.197	0.058
	Coefficient	**SE**	**t**	**Lower CI**	**Upper CI**	***p*-value**
Absolute latitude	0.0059	0.0032	1.843	0.0006	0.0125	0.048
Mangrove area	-0.0015	0.0010	-1.528	-0.0035	0.0005	0.060
Shape index	0.0095	0.0056	1.695	-0.0019	0.0209	0.051

1.4 DISCUSSION

Macroecological distribution patterns of nematode species richness was not explained by the richness of mangrove trees. Heterogeneity of mangrove forests is important in nematode community assembly at the local (Pinto et al. 2013; Sabeel and Vanreusel 2015) and regional scales (Fonseca and Netto 2015). Yet, the present study showed that at larger spatial scales nematode richness is not directed related to mangrove tree richness. This pattern contrasts with that of crabs and littorinid gastropods, which are both strongly associated with mangrove tree richness (Ellison 2008). Despite the lack of any relationship between nematode and mangrove richness, previous studies showed that nematode genus compositions differed among estuaries with and without mangroves (Fonseca and Netto 2015). Habitat type is considered important in shaping benthic metacommunities from local to global scales (Nagelkerken et al. 2008; Pinto et al. 2013; Song et al. 2017). In this sense, mangrove tree richness may increase the number of habitat niches for macrofauna

(Ellison 2008). However, data on mangrove meiofauna are generally restricted to local studies that do not cover all habitat heterogeneity. Nematode diversity in the upper-littoral zone where *Xylocarpus*, *Aegiceros*, *Heritiera*, *Acanthus*, and other mangroves tree species are distributed, are still poorly sampled and described. Therefore, the mangrove zones sampled in the studies that were included in our meta-analysis do not reflect the entire mangrove floristic diversity, or its full contribution towards structuring potential nematode richness.

Despite its relatively high explanatory power, there was no significant difference in nematode richness among biogeographic regions. Nematode species richness was not highest in the Central Indo-Pacific, even though this biodiversity hotspot has already been described for several other marine coastal taxa, and the region has been considered as a center of origin, overlap or accumulation of species (Renema et al. 2008; Bellwood and Meyer 2009). Biodiversity hot-spots such as the Central Indo-Pacific harbor and export species, but can also accumulate biodiversity from adjacent areas. Both hotspots and peripheral ecosystems benefit from this biodiversity feedback (Bowen et al. 2013) and the complexity of the biogeographical area where a mangrove forest is located seems determinant for its tree and associated fauna richness (Ellison 2008). This might not be necessarily true for nematode richness which seems more variable at smaller scales, probably due to their lower dispersal capabilities compared with macrofaunal invertebrates.

Latitude, rather than biogeographic region, was the main factor in structuring nematode richness at larger spatial scales. There was a significant correlation between nematode richness and latitude, with higher richness occurring at lower latitudes. The importance of latitude in structuring marine organisms has been reported for a variety of taxa and marine systems (Hillebrand 2004). These latitudinal patterns of distribution might be related to temperature gradients, which suggest the roles of regional environmental and climatic factors in structuring nematode richness at large scales (Song et al. 2017).

Local landscape moderators had a secondary role in structuring nematode richness, with total mangrove area and forest shape index accounting for 7.1% of the total variance explained by our multivariate model. Shape index can be used as a proxy of landscape complexity. The weak but negative correlation between nematode richness and mangrove forest area as well as the positive correlation with shape complexity indicates that landscape structure can be a potential driver of spatial

variation in nematode assemblages. In fact, the type of vegetation seems determining for nematode composition and structure in both marine and terrestrial environments (Fonseca and Netto 2015; Song et al. 2017). However, whether the spatial heterogeneity within the same vegetation type influences nematode richness still needs better appreciation.

The fact that there was no correlation between nematode richness and above-ground biomass of mangrove forests was rather unexpected. Apparently, mangroves with distinct forest biomass can support a similar average nematode richness. The rapid generation time of nematodes compared with the time needed for leaf degradation may generate this decoupling between nematode diversity and mangrove leaf litter (Gwyther 2003).

There were no significant effects of core volume or mesh size on average nematode richness. In our case, since methodological differences such as core volume or sieve size were not significant, the remaining heterogeneity might be either due to the relatively small number of observations or due to the intrinsic characteristics of studies that were not included in the model (e.g., environmental conditions at the local scale, differences in author's taxonomic accuracy, quality, and conservation of the sampled material). Despite the fact that meta-analysis is robust to intermediate sample sizes (e.g., $20 < k < 50$), and the confidence intervals generated are accurate (Hedges et al., 1999), incorporating more studies into future meta-analyses will increase model robustness and accuracy. Also, sharing of detailed local-scale data on abiotic factors rarely available in published studies (e.g., redox potential), as well the use of recurrent proxies such grain size and sorting, will enhance our understanding on how local and regional variations in environmental and biogeochemical conditions affect meiobenthic diversity and distribution.

In conclusion, our results contrast with biogeographic diversity patterns of highly-associated mangrove taxa and species from other marine ecosystems. Global-scale nematode diversity may have evolved independently of mangrove tree richness and is probably driven by regional and climatic factors. At local scales, nematode richness increased slightly with complexity of the mangrove landscape. Overall, for small-bodied taxa, latitude seems to overrule local factors and east-west biogeographic biodiversity patterns. This finding, therefore, has implications for patterns of meiofaunal species richness in a future world, where increasing ocean temperatures are driving range shifts of many species.

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SUPPORTING INFORMATION

Tab. S1 List of references used in nematode richness data compilation. (Ref.) references; (Est.) Estuaries; (lat.) Latitude in UTM; (long.) Longitude in UTM; (core) core volume in cm^3; (mesh) mesh size in µm; (n) number of samples in each study; (st) number of stations in each study; (mean) mean nematode richness between stations; (S) standard deviation of nematode richness between stations; (S^2) variance; ($1/S^2$) inverse variance; ($mean*1/S^2$) weighted mean nematode richness.

Code	Ref.	Est.	lat.	long.	core (cm^3)	mesh	n	st	mean	S	S^2	$1/S^2$	$mean*1/S^2$
1	Chen et al., 2012	Teluk Awar	1.67	110.48	73.59	32	21	7	11	8.3	68.8	0.015	0.16
2	Gee and Somerfield, 1997	Sungai Merbok	5.64	100.45	-	63	40	17	38	12.2	149.1	0.007	0.25
3	Somerfield et al., 1998	Sungai Merbok	5.64	100.45	21	63	16	4	29	6.0	36.0	0.028	0.81
4	Shabdin and Othman, 1999	Lok Kawi beach	5.87	116.03	210	32	10	5	33	6.0	36.0	0.028	0.92
5	Shabdin and Othman, 2008	Lok Kawi beach	5.87	116.03	210	32	40	5	20	5.1	26.5	0.038	0.75
6	Xuan et al., 2007	Can Gio/Khe Nhan	10.04	106.77	96.16	38	12	4	34	4.6	21.3	0.047	1.59
7	Mokievsky et al., 2011	Be River estuary	12.20	109.18	28.26	40	9	3	27	2.9	8.3	0.120	3.28
8	Chinnadurai and Fernando, 2007	Coleroon/Vellar	11.44	79.78	70.65	63	20	5	15	5.6	31.0	0.032	0.48
9	Ansari et al., 2014	Vellar	11.48	79.77	24.53	53	72	24	29	6.8	45.6	0.022	0.64
10	Alongi, 1987	Escape river	-10.97	142.67	33	45	6	2	18	6.0	36.0	0.028	0.50
11	Alongi, 1987	Claudie river	-12.82	143.35	33	45	6	2	21	6.0	36.0	0.028	0.58
12	Alongi, 1987	Lockhart river	-12.88	143.38	33	45	6	2	20	6.0	36.0	0.028	0.56
13	Decraemer and Coomans, 1978	Lizard Island/mang A	-14.65	145.47	-	-	1	3	31	3.8	14.3	0.070	2.14
14	Decraemer and Coomans, 1978	Lizard Island/mang B	-14.65	145.47	-	-	1	7	16	5.6	31.1	0.032	0.51
15	Alongi, 1987	Morgan/McIvor	-15.01	145.23	33	45	6	2	15	6.0	36.0	0.028	0.42
16	Alongi, 1987	Missionary Bay	-18.27	146.22	33	45	12	4	20	6.0	36.0	0.028	0.56
17	Alongi, 1990	Chunda Bay	-19.28	147.05	33	45	72	2	20	5.7	32.0	0.031	0.63
18	Hodda and Nicholas, 1985	Hunter River	-32.73	151.68	29.44	50	84	14	18	6.0	35.5	0.028	0.50
19	Hodda and Nicholas, 1986	Hunter River	-32.73	151.68	29.44	50	140	28	14	3.9	15.2	0.066	0.95
20	Nicholas et al., 1991	Clyde River	-35.73	150.14	17.00	50	20	4	18	4.7	22.3	0.045	0.78
21	Nicholas and Stewart, 1993	Clyde River	-35.73	150.14	19.87	50	25	5	19	2.2	4.7	0.213	4.09
22	Nicholas and Stewart, 1993	Candlagan Creek	-35.84	150.17	19.87	50	25	5	18	2.0	4.0	0.250	4.60

Tab. S1 Continued

Code	Ref.	Est.	lat.	long.	core (cm^3)	mesh	n	st	mean	S	S^2	$1/S^2$	mean*$1/S^2$
23	Gwyther, 2003	Barwon River	-38.28	144.50	-	53	48	1	21	6.0	36.0	0.028	0.58
24	Gwyther and Fairweather, 2002	Barwon River	-38.28	144.50	-	53	162	3	13	9.0	80.3	0.012	0.16
25	Gwyther and Fairweather, 2005	Barwon River	-38.27	144.47	-	53	150	3	26	9.4	87.9	0.011	0.30
26	Ólafsson, 1995	Maruhubi	-6.15	39.20	48	40	5	1	18	6.8	46.2	0.022	0.39
27	Ólafsson, 1995	Chukwani	-6.20	39.20	48	40	5	1	19	5.7	32.3	0.031	0.58
28	Ólafsson, 1995	Chwaka Bay	-6.18	39.42	48	40	5	1	22	7.0	48.5	0.021	0.45
29	Ólafsson, 1995	Muwanda	-5.92	39.22	48	40	5	1	12	7.6	57.7	0.017	0.20
30	Ólafsson et al., 2000	Muwanda	-5.92	39.22	42.5	40	35	7	11	4.0	15.9	0.063	0.67
31	Torres-Pratts and Schizas, 2007	Magueyes Island	17.97	-67.47	-	53	29	1	25	6.0	36.0	0.028	0.69
32	Pinto et al., 2013	Santa Cruz Channel	-7.77	-34.87	100	44	45	9	17	3.4	11.4	0.088	1.49
33	Netto and Gallucci, 2003	Ratones River	-27.48	-48.50	49.06	63	24	6	37	12.7	162.3	0.006	0.23
34	Fonseca and Netto, 2006	Laguna	-28.20	-48.63	31.4	63	60	15	15	6.0	36.0	0.028	0.42

Tab. S2 Omnibus test for heterogeneity (Q_T) and output results of the random-effects meta-analyses with the omnibus test (Q_M) for each single moderator tested and their respective *p*-values for Egger's regression test for funnel plot asymmetry. Relative amount of heterogeneity (i.e., between-study variance) in relation to the total unaccounted variability (I^2); Amount of variance (heterogeneity) accounted for by the moderators (R^2); Degrees of freedom (*df*); Test for residual heterogeneity (Q_E).

	Heterogeneity			**Mixed-effects**							**Egger's test**
	df	Q_T	*p*-value	I^2	R^2	*df*	Q_M	*p*-value	*df*	Q_E	*p*-value
Global Model	33	556.479	0.0001	-	-	-	-	-	-	-	0.0380
Moderators											
Marine Ecoregions	-	-		92.21	24.24	3	7.096	0.0689	30	384.975	0.001	0.0166
Longitude	-	-		94.23	0.00	1	0.109	0.7416	32	554.679	0.001	0.0382
Latitude	-	-		92.68	12.22	1	3.929	**0.0474**	32	437.341	0.001	0.0096
Mangrove richness	-	-		93.80	3.34	1	0.660	0.4166	32	516.222	0.001	0.0278
Biomass (AGB)	-	-		94.02	0.77	1	0.323	0.5698	32	535.489	0.001	0.0440
Mangrove area	-	-		93.87	2.59	1	0.012	0.9102	32	521.953	0.001	0.0335
Shape index	-	-		93.90	1.58	1	1.547	0.2135	32	524.585	0.001	0.0491
Sieve size	-	-		94.33	0.00	1	1.218	0.2698	30	529.553	0.001	0.0960
Core volume	-	-		93.57	0.00	1	0.448	0.5034	25	388.647	0.001	0.0736

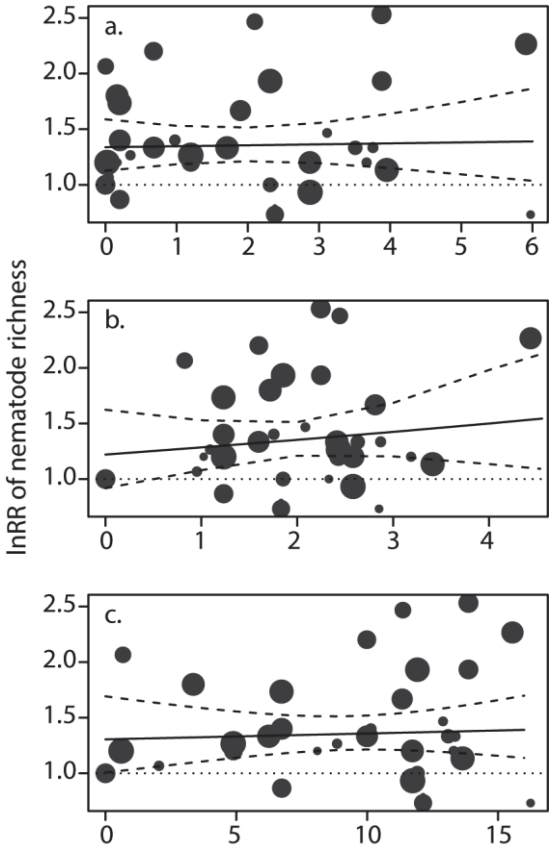

Fig. S1 Scatterplot of the lnRR of nematode richness of the individual studies plotted against the natural logarithm of mangrove forest area (a.), shape index (b.) and above-ground biomass (c.), respectively. Point sizes are drawn proportional to the inverse of the standard errors (i.e., more precise studies are shown as larger points). Solid line represents predicted values based on a mixed-effects model (with corresponding 95% confidence intervals).

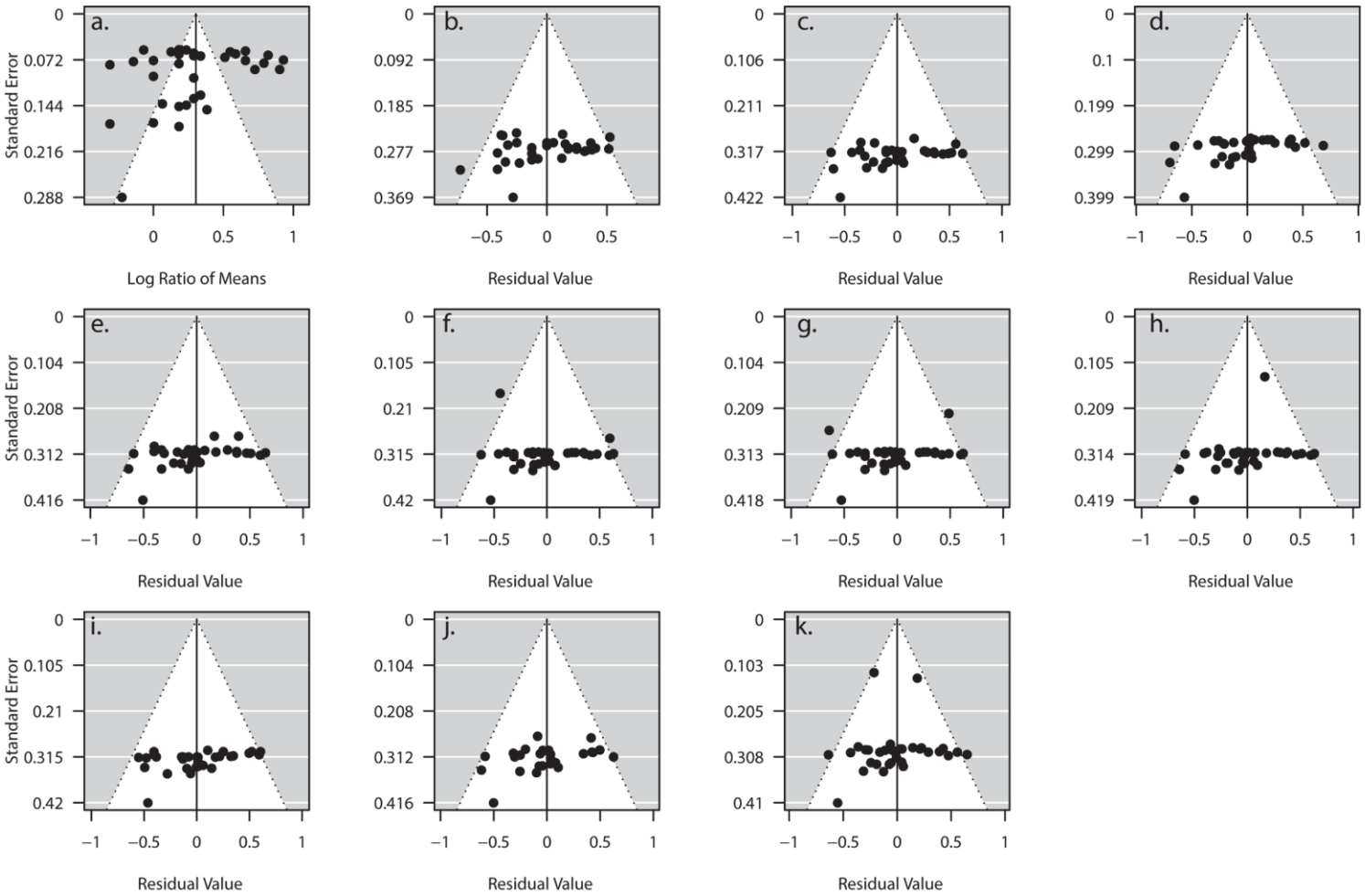

Fig. S2 Funnel plots of the effect estimates from individual studies against standard error of fitted univariate and multivariate random-effects meta-regression models. Outer dashed lines indicate the triangular region within which 95% of studies are expected to lie in the absence of both biases and heterogeneity. Metanalysis without any moderator (a.); marine ecoregions (b.); longitude (c.); absolute latitude (d.); mangrove richness (e.); above-ground biomass (f.); mangrove forest area (g.); shape index (h.); sieve size (i.); core volume (j.); multivariate meta-regression with absolute lat., cover area and shape index as continuous moderators (k.).

2 CHAPTER 2: METACOMMUNITY STRUCTURE AND SPATIAL VARIATION OF BENTHIC COASTAL ECOSYSTEM

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Abstract

We investigated the spatial patterns of nematode metacommunities along three nearby coastal environments. Mangroves, estuarine unvegetated tidal flats, and oceanic sandy beaches were sampled using a nested design composed by four spatial scales (150 km, 100 km, 100 m, 10 m). Using the elements of metacommunity framework (EMS), we accessed the type of metacommunities. Their patterns of spatial variation and the relative importance of spatial and environmental effects for the metacommunity structure were also investigated. We hypothesized that distribution of soft-bottom metacommunities will depend on the exposure to hydrodynamics. In mangroves, hydrodynamic energy is lower, diffusion and small-scale biogeochemical gradients are the predominant processes in structuring

community. This creates a mosaic of patches with different quality (e.g., food, competition, predation pressure) and nematode communities were structured by richness differences, showing a nested pattern. In unvegetated tidal flats, communities were structured by species turnover along environmental gradients (e.g., variation in grain size and sorting, salinity), having a Clementsian distribution associated to a combination of species sorting and patch-dynamics. In sand beaches the advective energy predominates, increasing connectivity and reducing the role of environmental filtering. Consequently, species were randomly distributed, and small-scale spatial variability was lower if compared with mangroves and tidal flats. Also, spatial component explained a smaller fraction of the variance in community structure if compared with other habitats. Communities at mangroves and tidal flats are structured by environmental filtering in a more niche-based dynamic, while communities at high-hydrodynamic sand beaches are mainly driven by mass-effect or neutral processes.

Keywords: Beta-diversity, EMS framework, Free-living marine nematodes, Meiofauna

2.1 INTRODUCTION

Species dispersal, habitat heterogeneity, and ecological neutrality determine the biodiversity pattern of metacommunities and the preponderant mechanisms influencing its dynamics (Logue et al. 2011, Brown et al. 2017). Therefore, the roles of niche and and neutral dynamics in metacommunity assembly can be evaluated by understanding how metacommunities are distributed in the space and how they change through time. Different approachs can be used to explore metacommunity dynamics. Tow recurrent ways are the mechanistic and the pattern-based. The mechanistic approach is based on spatially mediated models (i.e., patch dynamics, species sorting, mass effects, and neutrality) and their underlying mechanisms, such as dispersal, biotic interactions, or responses to abiotic environmental characteristics (Leibold et al. 2004a, Cottenie 2005). On the other hand, the pattern-based approach (Leibold and Mikkelson 2002) called *elements of metacommunity structure* (EMS), evaluates the fit of empirical data to previous-known models (e.g., checkerboard,

nested, evenly spaced, Gleasonian, Clementsian, and their respective quasi-variations) (Presley et al. 2010).

Metacommunity organization is not static and might show a high degree of variability in space and time due to changes in the environmental filtering and species dispersal rates (Datry et al. 2016, Brown et al. 2017). In fact, the distinction among metacommunity types seems related to differences in their patterns of variation over multiple spatial scales. Dispersal limitations generally affect beta-diversity at large-scales, whereas environmental filtering operates at smaller-scales (Declerck et al. 2011, Dümmer et al. 2016). Likewise,Clementsian structures are more likely to be generated by large-scale variations on environmental conditions, whereas nestedness increases when small-scale spatial variability is higher (Valanko et al. 2015). These observations suggest that metacommunity structure is dependent on the scale and may change in response to variations in the magnitude of environmental gradients. The EMS approach has been recently applied to understand the spatio-temporal dynamics of invertebrate communities from streams (Datry et al. 2016), lakes (Dümmer et al. 2016), and coastal ecosystems (Valanko et al. 2015). Particularly for coastal ecosystems, where habitat limits are not always easy to recognize, the EMS approach can be very informative, helping us to understand why metacommunities differ, and which are the mechanisms controlling its dynamics (Presley et al. 2010, Gascón et al. 2016).

Particularly for the marine benthos, habitats can be structured by advection (e.g., oceanic sandy beaches) or by diffusion processes (e.g., mangroves, and non-vegetated estuarine tidal flats) depending on their exposure to hydrodynamic energy (Fig.1). In sandy beaches, waves and currents are constantly resuspending and transporting sediments, and invertebrate communities are mainly structured by physical/advective forces (Mermillod-Blondin and Rosenberg 2006). Mangroves and estuarine tidal flats on the contrary are less exposed, and biogeochemical/diffusion gradients within the sediment are the main structuring forces (Mermillod-Blondin and Rosenberg 2006, Vieira and Fonseca 2013). Following the current theoretical framework, we hypothesize that spatial pattern of soft-bottom metacommunities from coastal ecosystems will depend on the exposure to hydrodynamics (Fig.1).

To test this hypothesis, we used free-living nematode communities as a model for investigating the types of metacommunities (by means of EMS analyses) from three distinct coastal environments with different hydrodynamic regimes (i.e.,

mangroves, estuarine unvegetated tidal flats, and oceanic sandy beaches) at four different spatial scales (150 km, 100 km, 100 m, 10 m). Specifically, we expect that (i) mangrove metacommunity structure will present a distribution pattern constrained by geographical distance and environmental conditions. Small-scale environmental heterogeneity creates a mosaic composed by patches with different quality (e.g., food, oxygen, competition, and predation pressure) which might affect richness therefore generating nested distributions; (ii) In unvegetated tidal flats the importance of small-scale heterogeneity will be lower compared to mangroves since they are more exposed to tidal currents witch redistribute food and shape sediment's bed with more intensity, therefore reducing geographical constraints. We expect community will be marked by a pattern of species substitution (i.e., turnover) along environmental gradients acting inside estuarine areas, such as variation in grain size and sorting, salinity, and exposure. Thus, we predict metacommunity from unvegetated tidal flats will fit into Gleasonian or Clementsian patterns; and (iii) Metacommunity structure from exposed sand beaches will present a random pattern of distribution generated by the high hydrodynamic energy and passive dispersal, which prevent species extinctions within beach local communities.

We have also investigated their pattern of spatial variation over the four hierarchical spatial scales (100 km, 100 m, 10 m), and the relative importance of spatial and environmental constraints for the metacommunity structure. We hypothesize to find higher turnover at protected sites, while turnover will be lower in exposed habitats. Following the same rationale, the relative contribution of each spatial scale to the total variance of the community will vary among habitats: exposed habitats will have higher variation at larger scales (100 Km), due to homogenization of the fauna at the small-scale; whereas at protected habitats variation at smaller scales (10 and 100 m) will be higher than small-scale variability observed at sand beaches, since species are dispersion limited and habitat is highly heterogeneous. In addition, based on variance-partitioning analysis we also expect that exposed metacommunities will be largely explained by the environmental variables (species dissimilarity increasing with ecological heterogeneity) and the spatial component will be less important. In contrast, a larger contribution of the spatial component (species dissimilarity increasing with spatial distance) will be observed in protected habitats, where species dispersion is limited.

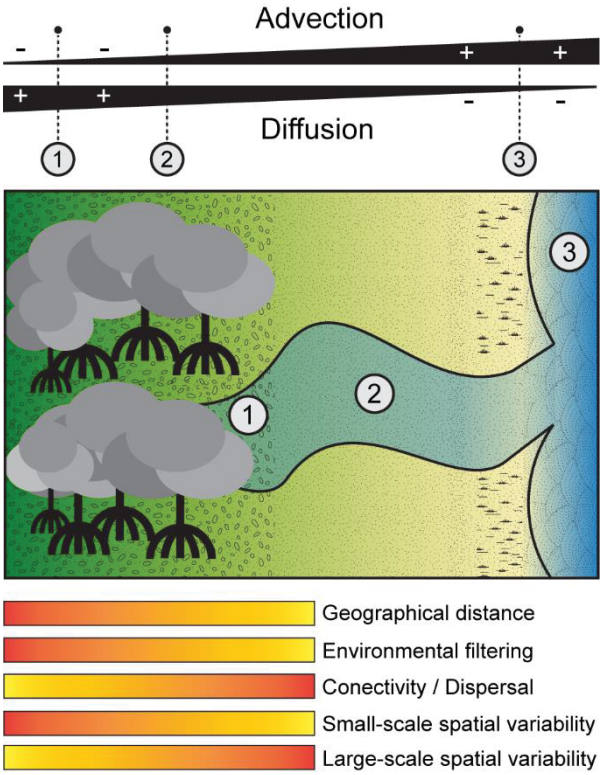

Fig. 1 Theoretical framework of the hydrodynamic energy gradient and environmental drivers of meiobenthic metacommunity structure in coastal ecosystems. Numbers are indicating the presupposed degree of ecosystem's exposure. Intertidal mangrove areas (1); Unvegetated tidal flats (2); Oceanic sand beaches (3). The relative importance of ecological mechanisms and spatial patterns of variation along the gradient are given by color bars (i.e., importance increasing from yellow to red).

2.2 METHODS

2.2.1 Study area

Our study was done in the subtropical coast of São Paulo (Fig. 2), Brazil (between 25° 3'36.83" and 23°20'16.02" S and 47°55'45.53" and 44°53'19.11" O). The region is located in the São Paulo Bight sector of the Southern Brazilian continental shelf, which is the limit between the transition zone influenced by sediments from Río de La Plata, and the Cabo Frio/Cabo de São Tomé sector where morphodynamics is less influenced by the confluence between Brazil and Malvinas currents (Mahiques et al. 2010). The littoral zone is composed by several estuarine systems dominated by mangrove forests, which are intercalated by barrier islands and sandy beaches of different sizes and orientation (Schaeffer-Novelli and Cintrón-Molero 1990).

2.2.2 Sampling design

One sampling campaign was conducted using a nested sampling design of four spatial scales at three different coastal ecosystems (mangrove, unvegetated tidal flats, and oceanic sandy beaches) (Fig. 2). Ecosystems were sampled, in four different locations (100 km distant), at the littoral zone of São Paulo, Brazil SE (Fig. S1). Two plots hundreds of meters distant (100 m) were randomly established inside the four different locations. At each plot, four sediment samples tens of meters distant (10 m) were taken with a 2.5 cm diameter core, down to a depth of 5 cm for nematode assemblage analysis (supplementary material Fig. S1). Four other sediment samples (2 g each) were collected for chloroplastic pigment content and granulometric analyses (10 g each). Samples for meiofauna analyses were fixed in 4% formaldehyde. Sediment samples for the photosynthetic pigments and grain size analyses were stored in a cooler in the field and frozen at −20 °C in the laboratory.

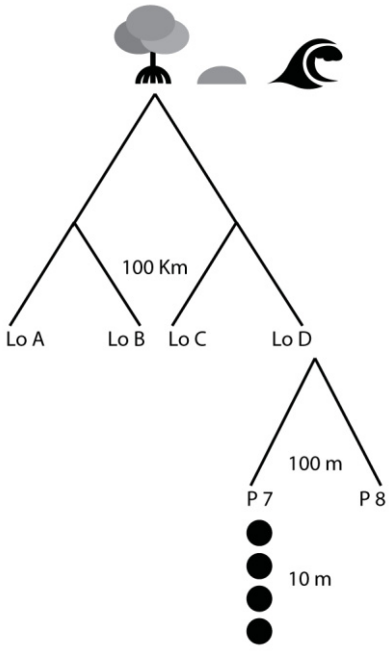

Fig. 2 Nested sampling design used in the study. Locations 100 Km distant (Lo) are respectively Cananéia (A), Juréia (B), Boracéia (C), and Ubatumirim (D).

2.2.3 Samples processing

Samples for nematode assemblage analysis were washed through a 45 µm mesh sieve. After washing, the flotation method with colloidal silica solution (Ludox TM-50) adjusted to a specific gravity of 1.18 was used to separate organisms from the sediment (Heip et al. 1985). All nematodes were counted on a Dolffus plate under a stereoscopic microscope; For each sample, 20% of total nematodes were picked (following Vincx 1996), evaporated to anhydrous glycerol and mounted on permanent slides for identification. A lowest and highest limit of 100 and 250 nematodes were established (i.e. when the total of nematodes was lower than 100, all individuals were picked for identification and when the total was higher than 1250, only 250 individuals were taken). Nematodes were identified to genus level (Warwick et al. 1998) and further separated into species or putative morphospecies.

Pigments were extracted with 10 ml acetone (100%) for 24 h in the dark at 4 °C. The extract was centrifuged at 4000 rpm for 5 min. Pigments were analyzed from absorbance spectra at 665 nm and 750 nm before and after acidification with HCL through a digital spectrophotometer. Concentrations of pigments were estimated using the equations of Lorenzen (1967). Sediment granulometry was determined by sieving dried samples and total organic content by difference in dry weight after combustion for 4 hours at 550°C. The redox potential was measured at the sediment surface (ca. 1 cm depth) using a Hanna Instruments HI 991003.

2.2.4 Data analysis

Elements of metacommunity structure EMS

The elements of metacommunity structure (EMS) were estimated and interpreted according Leibold and Mikkelson (2002) and Presley et al. (2010). The EMS approach tests the fit of empirical data to several types of metacommunity structure, such as checkerboard, nested, evenly spaced, Gleasonian, Clementsian, and random (Leibold and Mikkelson 2002, Presley et al. 2010). These patterns can be described by three properties of metacommunity structure: coherence, turnover, and boundary clumping. The metrics were calculated from a presence – absence matrix, with sites as rows and species presence/absence as columns. The interaction

matrix was ordinated via reciprocal averaging. The scores obtained from ordination can then be related to environmental or spatial variables (Presley et al. 2010). Coherence was measured by comparing the number of observed absences in the ordination matrix to the number of absences in randomized null matrices. A smaller number of embedded absences (Abs) than expected by chance indicates positive coherence, while many absences (i.e., absence is significantly larger than expected by the null models) indicates negative coherence. Significant positive coherence can be related to nestedness, evenly spaced gradients, Gleasonian or Clementsian gradients (Leibold and Mikkelson 2002). On the contrary, significant negative coherence indicates a checkerboard distribution, whereas a non-significant coherence suggests a random metacommunity and there is no need to proceed with other tests. Further analysis presupposes that community matrices have a positive coherence. Turnover was measured as the number of times one species replaced (Repl) another. Significantly negative turnover refers to nestedness, whereas significantly positive turnover (i.e., Repl is significantly larger than expected by chance) indicates evenly spaced, Gleasonian or Clementsian metacommunities. Significant positive coherence combined with a nonsignificant turnover can be interpreted as a quasi-structure (Presley et al. 2010). The evenly spaced, Gleasonian and Clementsian metacommunity types can be distinguished subsequently based on the index of boundary clumping (Leibold and Mikkelson 2002). Boundary clumping was analyzed using Morisita's *I* dispersion index. Statistical significance was obtained by comparing the observed range boundary of samples against a chi-square distribution. When the Morisita's dispersion index is not different from 1, the range boundary is randomly distributed, which fits with a Gleasonian metacommunity structure. A Morisita's *I* significantly higher than 1 indicates a clumped range boundary (i.e., Clementsian metacommunity) and a value significantly lower than 1 indicates a hyperdispersed range boundary (i.e., evenly spaced metacommunity). Coherence (Abs) and turnover (Repl) indices were tested using the fixed-proportional null model "r1", where row sums are fixed (i.e., the species richness of each site was maintained), but column marginal frequencies (i.e., species frequencies of occurrence) were used as probabilities. Random matrices were produced based on 999 simulations and statistical significance of Abs and Repl was assessed by comparing the observed outcomes to the distribution of outcomes derived from the randomizations. Elements of metacommunity structure were evaluated based on axis

1 of reciprocal averaging. EMS analyses were done using the R package ‘metacom’ (Dallas 2014) in the R environment (version 3.3.3, R Development Core Team 2017).

2.2.5 Spatial variation on metacommunity structure

To evaluate the significant differences in metacommunity structure between different ecosystems across multiple spatial scales, nested permutational analyses of variance (PERMANOVA) were performed using ‘adonis’ function of the R package ‘vegan’ (Oksanen et al. 2016). *EMS* scores were used as dependent variable. Ecosystems were treated as fixed term composed by three distinct levels (i.e., mangrove, unvegetated tidal flats and oceanic beach sites), while locations (100 Km distant) and the subsequent nested spatial scales (100 m, 10 m) were considered random factors (see supplementary material Fig. S1).

2.2.6 Variance-partitioning and the structuring gradient associated with ecosystem’s characteristics

Overall nematode community composition in mangroves, tidal flats, and sandy beaches were analyzed using variation partitioning via distance-based RDA (Peres-Neto et al. 2006) in the R package ‘vegan’ (Oksanen et al. 2016). Bray-Curtis dissimilarities of the nematode fauna were used as beta-diversity metric. RDA analyses were performed using principal coordinates of neighborhood matrices (PCNM) (Dray et al. 2006). Sediment grain size and sorting, organic matter, chlorophyll-*a*, phaeopigments, and redox potential were included as environmental descriptors. Significant relationships between the species and environmental variables support the hypothesis of environmental filtering, whereas significant spatial patterns can support either neutral mechanisms or controls via spatially structured environmental gradients (Legendre 2008). Hypotheses are not mutually exclusive and community beta-diversity is generally driven by a combination of both, environmental and dispersal-based processes (Legendre 2008, Brown et al. 2017).

We use a least square multiple regression with a backward stepwise selection to investigate the association between the EMS site scores obtained from the reciprocal averaging ordination and a set of explanatory variables, including sediment

grain size, and sorting, organic matter, chlorophyll-*a*, phaeopigments, and redox potential. The most parsimonious model was chosen based on the lowest Akaike Information Criteria (AIC). Backward regressions were performed using the R package 'MASS' (Venables and Ripley 2002). The relative importance of explanatory variables for the linear model was calculated through the partitioning of R^2 by averaging over orders using the R package 'relaimpo' (Grömping 2006).

2.3 RESULTS

2.3.1 Elements of metacommunity structure EMS

The overall nematode metacommunity from coastal ecosystem was significantly coherent (z = 5.87, p < 0.001). Also, metacommunity presented more species replacement (Repl) than expected by chance (z = -81.11, p < 0.001) and species range boundaries were clumped (Morisita's I = 22.9, p < 0.001). This suggests a Clementsian structure, where nematode species respond to a structuring gradient as a group (Table 1). In this case, nematode metacommunity was structured mainly by niche effects associated with habitat characteristics. Local communities from mangroves, estuarine tidal flats, and sandy beaches were composed by different species groups that replaced each other along the coastal seascape (Fig S2a).

Table 1 Summary results of the elements of metacommunity analysis (EMS) for the overall model including all habitats as well as for each habitat. Simulated mean (Sim), embedded absences (Abs), and replacement (Rep). Metacommunity structure: nested pattern with clumped species loss (NCSL); and random pattern (Random). Mangrove: Mang.; Estuarine tidal flat: Est.; Sandy beach: Beach.

	Coherence			Turnover			Clumping		Structure
	Sim	Abs	Z	Sim	Repl	z	*df*	Morisita's	
Overall	9585	9158	5.87***	707411	161^{E-4}	-81.11**	117	22.9***	Clementsian
Mang.	1798	1759	1.98*	64896	405^{E-2}	10.91***	74	28.2***	NCSL
Est.	1425	1364	2.65**	37555	408^{E-2}	-2.24*	59	21.6***	Clementsian
Beach	1373	1367	0.41	48576	374^{E-2}	4.66***	60	31.7***	Random

Significance codes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Coherence was significantly positive in mangrove and estuarine tidal flats (Table 1). In addition, mangrove metacommunity showed less species replacement (Repl) than expected by chance (z = 10.91, p < 0.001) and clumped range

boundaries (Morisita's *I* = 28.2, p < 0.001), characterizing a nested pattern with clumped species loss (Table 1).

Species replacement of the metacommunity from tidal flats was higher than expected by chance (z = -2.24, p < 0.05), and species range boundaries were also clumped (Morisita's *I* = 21.6, p < 0.001) suggesting a Clementsian structure (Table 1). Species groups replace each other along and within the locations (Fig. S2c).

There was no significant coherence in the sandy beach metacommunity structure, which suggests a random pattern where species were not structured by a major gradient (Table 1). In addition, turnover (Repl) of the sandy beach metacommunity was lower compared to that observed for mangrove and estuarine tidal flat metacommunities (Table 1).

2.3.2 Spatial variation on metacommunity structure

The spatial variation in metacommunity structure of the coastal ecosystem was significantly higher among 100 Km, 100 m and habitats. Habitats contributed with 31.3 % of the total variability, while 100 Km, 100 m contributed with 21.3 % and 22.9 %, respectively (Table 2). The spatial variation on metacommunity structure of protected habitats (i.e., mangrove and estuarine tidal flats) was significantly higher at 100 m (Table 2). Small scale (10 m) accounted for 49.8 % of total variability in mangrove metacommunity structure and 28.9 % in estuarine metacommunity. Spatial variation on metacommunity from exposed oceanic beaches was significantly higher among 100 m, 100 Km. In this habitat, small scale (10 m) accounted with 29.4 % of total variability, whereas larger scales (100 m and 100 Km) contributed with 70.5 % of total variability in metacommunity structure (Table 2).

Table 2 Multivariate permutational analyses of variance PERMANOVA for Overall, Mangrove, Unvegetated tidal flat and Beach metacommunity structure. Analyses were based on Bray-Curtis dissimilarities. df = degrees of freedom, MS = mean squares, (n=4).

		Overall			
	df	MS	*Pseudo-F*	CV	CV%
Ha	2	58014.0	4.497	1409.8	31.3***
100 km	9	12900.0	2.467	959.9	21.3***
100 m	12	5227.8	4.774	1033.2	22.9***
Res (10 m)	72	1094.9	-	1094.9	24.3
Total	95	-	-	4496.9	-
		Mangroves			

	df	MS	*Pseudo-F*	CV	CV%
100 km	3	8697.8	1.714	453.1	16.5
100 m	4	5073.3	3.709	926.4	33.7***
Res (10 m)	24	1367.6	-	1367.6	49.8
Total	31	-	-	2747.1	-
		Tidal flats			
	df	MS	*Pseudo-F*	CV	CV%
100 km	3	16023.0	2.924	1318.0	38.4*
100 m	4	5478.7	5.528	1121.9	32.7***
Res (10 m)	24	990.9	-	990.9	28.9
Total	31	-	-	3430.8	-
		Sand beaches			
	df	MS	*Pseudo-F*	CV	CV%
100 km	3	8697.8	1.714	1110.8	36.2*
100 m	4	5073.3	3.709	1054.7	34.3***
Res (10 m)	24	1367.6	-	903.5	29.4
Total	31	-	-	3069.1	-

Significance codes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

2.3.3 *Variance-partitioning and the structuring gradient associated with habitat characteristics*

Environmental heterogeneity alone explained 35 % of the variance in overall nematode metacommunity structure, while spatial effects had a minor contribution (Fig. 3a). In mangroves and tidal flats, environmental heterogeneity and spatial distance explained similar proportions of the variance in community structure, although unexplained variation was higher at mangroves (Fig. 3b, 3c). Also, the spatial component explained a smaller fraction of the variance in the metacommunity structure at sandy beaches if compared with other habitats (Fig.3c).

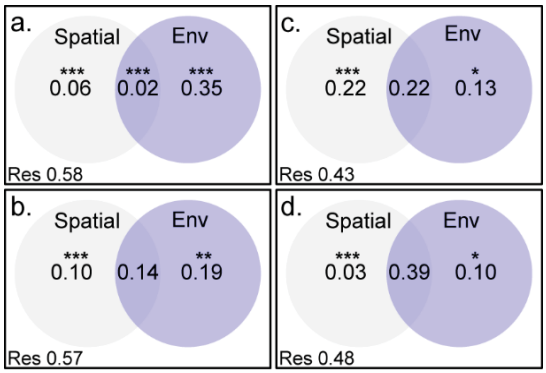

Fig. 3 Venn diagrams for variance-partitioning of Bray-Curtis dissimilarities among spatial and environmental components, p-values for spatial and environmental fractions were obtained through 999 permutations using partial CAP analyses. Overall metacommunity structure (a.), Mangrove (b.), Tidal flat (c.) and Sandy beach (d.)

metacommunities. Significance codes: *P < 0.05; **P < 0.01; ***P < 0.001.

In mangroves, fauna was mainly structured by organic matter content and heterogeneity in grain size and sorting (Fig.4a). In tidal flats, nematode species distribution was associated with chlorophyll-*a* and phaeopigment contents (Fig.4a). Nematode communities inhabiting sandy beaches were associated with well selected sediments, with relatively low food content and structured by asymmetry in grain size. Sandy beaches with coarse sediments hosted slightly different communities from those observed in fine and very fine sand beaches (Fig.4a). Consequently, nematode species composition differed among mangroves, tidal flats, and sandy beaches (Fig. 4b).

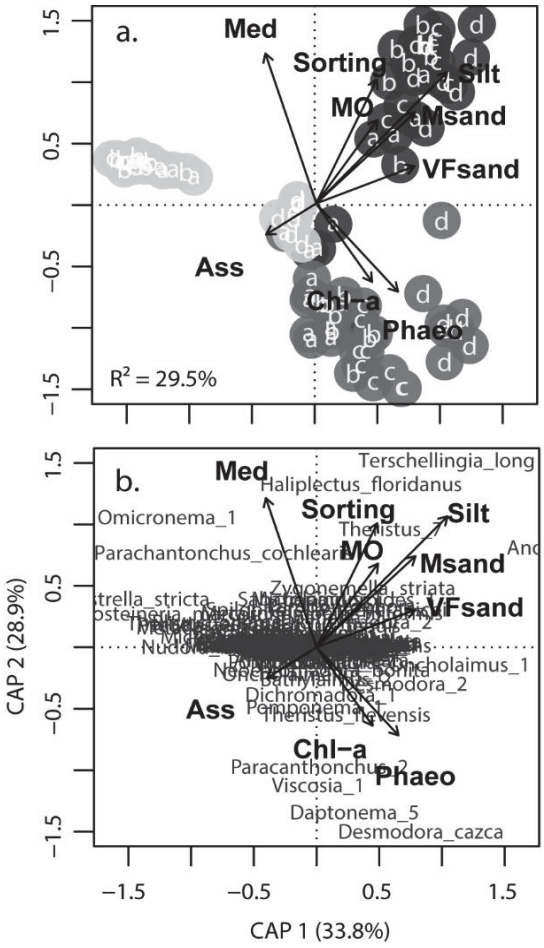

Fig. 4 Constrained Analysis of Principal Coordinates (CAP) based in the variance-partitioning of Bray-Curtis dissimilarities on nematode metacommunity among costal ecosystems. Mangrove (dark gray), Tidal flat (gray), and Sandy beach (light gray). Codes inside circles represent locations (10 Km). Cananéia (a), Boracéia (b), Juréia (c), and Ubatumirim (d).

Environmental variables explained a significant portion of the variance in scores obtained from EMS analysis (R^2=81.3%, p<0.001). The best regression model according to a backward stepwise selection included very coarse sand, silt, phaeopigments, sorting, and fine sand as significant explanatory variables (Table 3, supplementary material Fig. S3).

Table 3 Summary results of the least squares multiple regression testing the relationships between elements of metacommunity (EMS) site scores and changes on ecosystem's characteristics. Coefficients are shown with their standard deviation (n = 96). Proportion of variance explained by model (R^2) and relative importance of explanatory variables are shown in normalized to sum percentages (i.e., R^2 represent 100% of variance explained).

	df1	*df2*	*F*	R^2
	5	90	77.41	81.13***
	Coefficients		t-value	% of R^2
Sorting	0.002 ± 0.001		5.995	10.1***
Very coarse sand (%)	-0.148 ± 0.021		-7.137	32.0***
Fine sand (%)	-0.349 ± 0.059		-5.928	8.0**
Silt (%)	-0.139 ± 0.018		-7.773	38.3***
Phaeopigments	-0.021 ± 0.007		-3.191	11.2**
(Intercept)	7.981 ± 1.015		7.865	- ***

Significance codes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

2.4 DISCUSSION

Our results indicated nematodes were mainly structured by environmental filtering along the coastal plain. The transition between terrestrial/freshwater and marine ecosystems creates a complex and dynamic environment resulting in a highly heterogeneous landscape, which is more likely explained by a combination of mechanisms (Leibold and Loeuille 2015, Brown et al. 2017). Despite being adjacent environments with a strong interrelationship, mangroves, tidal flats, and sandy beaches hosted nematode communities with different species composition. This support the current notion that under strong environmental gradients metacommunities are not random-distributed (Gascón et al. 2016). Habitats contributed with 31.3 % of the total variability in overall nematode community. In variance partitioning analysis, environmental heterogeneity alone contributed with 35 % of the variance explained, while spatial effects had a minor contribution.

Morphodynamics and salinity gradients affect metacommunity organization shaping discrete communities with distinct life-histories and osmotic tolerances (Heino et al. 2015, Valanko et al. 2015). Therefore, the overall Clementsian structure of metacommunity support the idea that nematode communities were structured by species turnover along the transition gradient between freshwater and marine environments.

Benthic communities from different habitats were structured by different mechanisms depending on the hydrodynamic energy of the environment, allowing the emergence of different patterns of metacommunity organization. Freshwater invertebrate and marine metacommunities for example, are strongly influenced by direct and indirect effects of hydrodynamics (Vanschoenwinkel et al. 2007, Datry et al. 2016). Environmental heterogeneity and connectivity among habitat patches are determined by circulation patterns (Vanschoenwinkel et al. 2007, Yeh et al. 2015), and metacommunity shows Clementsian patterns at intermediate connectivity, but when connectivity is high, metacommunity shows random patterns (Yeh et al. 2015).

In our study, low-energy mangroves and tidal flats hosted communities more structured by niche-based dynamics, in a combination of species sorting and patch-dynamics (Vanschoenwinkel et al. 2007, Leibold and Loeuille 2015). In sandy beaches, on the other hand, high hydrodynamic energy increases connectivity in a more mass-effect and neutral-based dynamic.

Mangrove muddy sediments are poorly sorted, rich in organic matter content and less oxygenated due to the low hydrodynamic energy (Vieira and Fonseca 2013). Also, engineering (e.g., bioturbation, biodifusion) increases local heterogeneity. As a result, biogeochemical and diffusion processes increase in importance (Mermillod-Blondin and Rosenberg 2006). Hence, small-scale spatial variability in nematode assemblages was higher in mangroves compared to more hydrodynamic sandy beaches, generating distinct microhabitats (Netto and Gallucci 2003, Pinto et al. 2013). In addition, variability among mangrove forests (100 and 150 km distant) and fragments (100 m distant) were significant, and metacommunity presented a nested pattern of distribution. Thus, our results suggest that variation in patch quality drive nematode species richness. Differences in the size and complexity of drainage basins and mangrove forests, as well variations in tidal amplitude might affect richness at large-scales. Despite this, the spatial component explained a significant amount of variation in community structure, which suggest

nematode distribution is also influenced by dispersal constraints, mainly at larger-spatial scales (Declerck et al. 2011, Dümmer et al. 2016). Thus, the predominance of environmental filtering does not eliminate the contribution of spatial effects (Legendre 2008) and both had complementary roles in structuring nematode richness at mangroves.

Environmental filtering seems to overrule the effects of geographical distance at tidal flats, and dispersal rates are less important than the environmental gradient in structuring nematode communities. Nematodes presented a clementsian pattern, and ecosystem is characterized mainly by turnover differences. Environmental and spatial effects had similar contributions, which suggests that spatially structured environmental gradients might drive community structure (Legendre 2008, Gascón et al. 2016). Variation inside the same estuarine system may be related to interspecific differences in physiology (e.g., tolerance to salinity or tidal exposure), but also, with physical barriers generated by density gradients and the presence of maximum turbidity zones, which might limit connectivity and species overlap between inner and outer estuarine areas (Valanko et al. 2015). Also, small-scale variations in sediment texture and oxygen, as well in the amount and quality of food (e.g., refractory organic matter vs. labile food) among sediment patches can affect nematode fauna distribution (Vieira and Fonseca 2013, Brustolin et al. 2014). Consequently, the interaction between species sorting and patch-dynamics most probably drive metacommunity organization at tidal flats.

Conversely, mass-effect mechanisms likely drive nematode distribution at sandy beaches, which results in lower dominance and higher occurrence of rare species compared to mangrove and tidal flats. Hence, the importance of environmental filtering is lower. Metacommunity was randomly distributed, which means species have independent responses to multiple environmental gradients. In this case, geographical distance (i.e., spatial component) explained a lower amount of variability in community structure compared to the other ecosystems. Habitat homogenization and connectivity increases due to the high hydrodynamism (Yeh et al. 2015), allowing species coexistence and reducing the effects of competition/predation. At large-scales (100 km) both, spatial and environmental effects influenced nematode distribution. Aside dispersal limitations, beach orientation and morphodynamics, as well the characteristics of nearby ecosystems can affect species composition.

Here, we clearly demonstrated that environmental variability shape metacommunity organization at heterogeneous landscapes. Coastal ecosystems have its own characteristics and dynamics, and nematode community structure varied in response to advection energy, hence beta-diversity among ecosystems is higher. Conversely, variation within coastal ecosystems (i.e., mangroves, unvegetated tidal flats, and sandy beaches) was always higher at large-scale 100 km, compared to variation at 100 and 10 m, and small-scale spatial variability decreases in importance at sandy beaches where hydrodynamism is higher. Our results support the notion that niche and dispersal effects act simultaneously at different spatial scales, and the mechanisms shaping metacommunity organization are dependent on ecosystem's characteristics.

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SUPPLEMENTARY MATERIAL

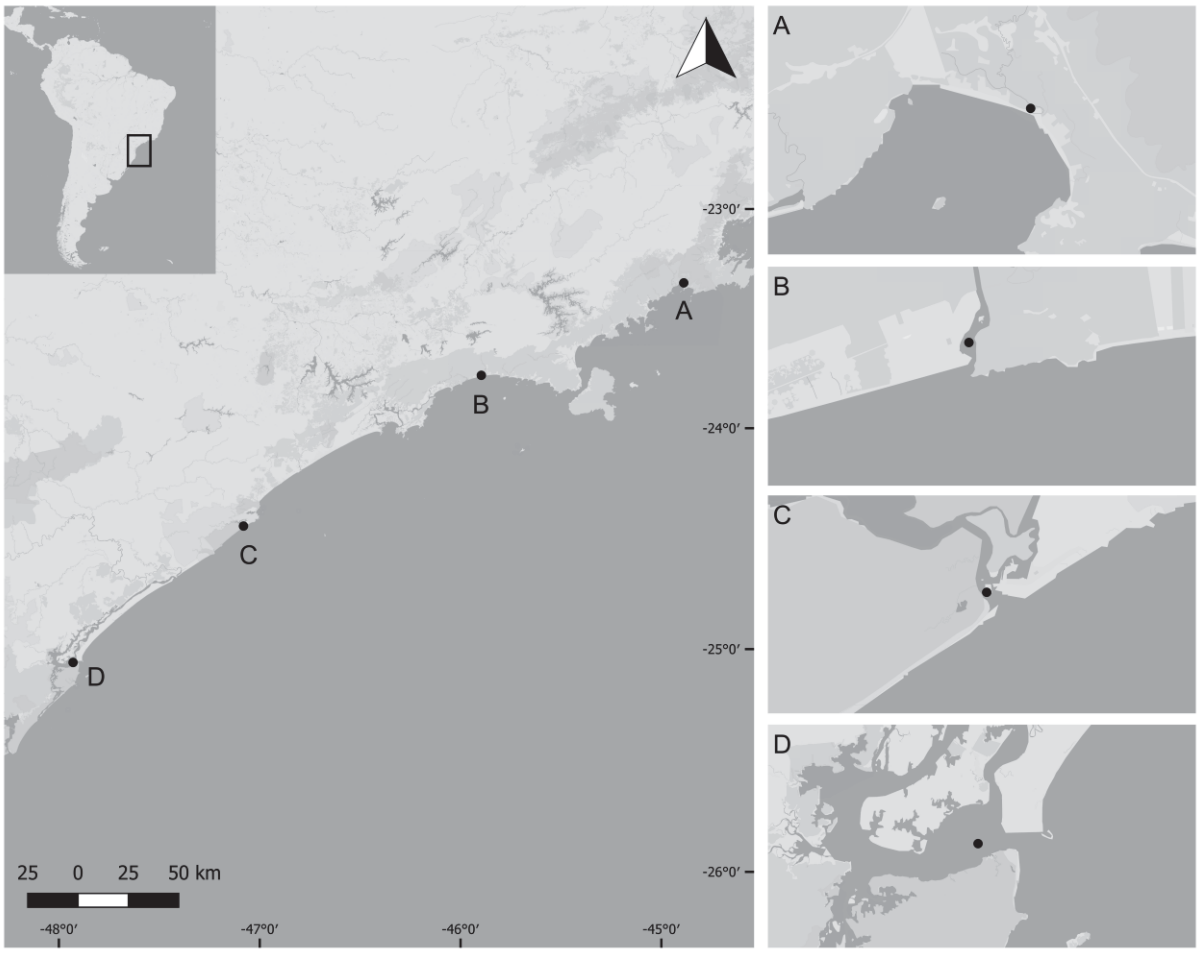

Fig. S1 Map of the study area at the coast of São Paulo, SE Brazil. Points represent the locations sampled, Ubatumirim (a), Boracéia (b), Juréia (c), and Cananéia (d).

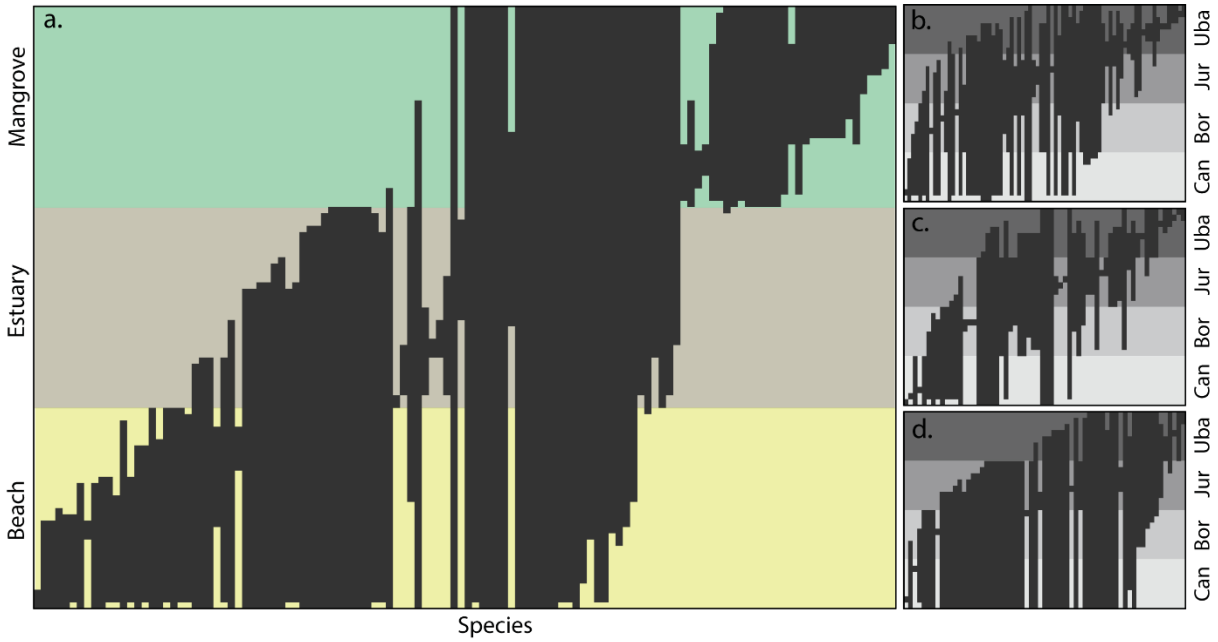

Fig. S2 Elements of metacommunity (EMS) site by species ordered matrices. Overall metacommunity structure (a.), Mangrove metacommunity (b.), Estuary metacommunity (c.) and Beach metacommunity (d.).

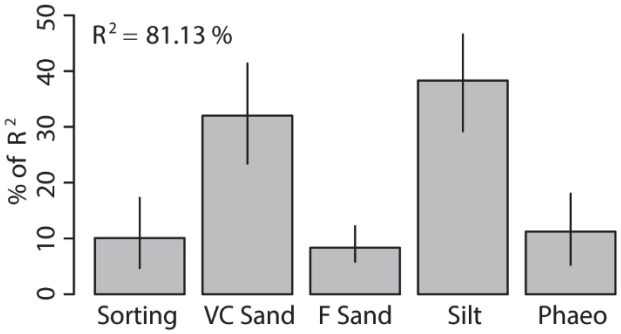

Fig. S3 Relative importance of environmental variables for elements of metacommunity (EMS) site scores. Deviation bars are 95 % bootstrap confidence intervals. Relative importance of explanatory variables is shown in normalized to sum percentages (i.e., R^2 represent 100% of variance explained).

3 CHAPTER 3: METACOMMUNITY VARIABILITY OVER MULTIPLE TIME-SCALES: DOES DAILY VARIATION MATTERS?

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Abstract
Despite the numerous literature demonstrating the existence of small-scale temporal variability in the distribution of invertebrate fauna, we still give little attention to the consequences of ignoring it when large-scale temporal dynamics are investigated. Here, we applied a hierarchical sampling design composed by four temporal scales (seasons, months, weeks, and days) to quantify the temporal variation of nematode

communities at two different habitats (estuarine tidal flats and sand beaches). Our aim was answering the following questions: (1) Are short-term temporal variations in community structure and composition greater than variation among seasons? (2) Does the temporal dynamics of nematode communities differ among habitats? (3) Is the temporal variation in community structure higher at more hydrodynamic sand beaches if compared with more protected tidal flats? We observed that short-term temporal variability has equal or more importance than seasonal environmental variations for community structure of small invertebrates. Environmental filtering was determinant for nematode metacommunity structure, and habitats hosted local communities with different temporal dynamics. Variation among days were higher at estuarine tidal flats, whereas variation among weeks had more importance for communities inhabiting sand beaches. Sand beaches are more variable, the advection energy increases dispersal/connectivity preventing local species extinctions and increasing turnover in space and time. These highlights the need to evaluate variability over multiple temporal scales to fully understand the effects of environmental and ecological processes in the structure and dynamics of benthic communities.

Keywords: *Seasonal dynamics; Small-scale variability; Sand beach; Tidal flat; Benthos; Meiofauna*

3.1 INTRODUCTION

Understand the variability across different spatial and temporal scales is a prerequisite for conserving and managing ecological communities (Whittaker et al. 2001, Chave 2013). The emergent effects of long-term climate changes and human disturbances on biodiversity have impelling scientists to better understand ecological and evolutionary mechanisms of metacommunity assembly (Urban et al. 2012). In heterogeneous landscapes, environmental variability, spatial constraints, and species interactions (e.g., competition, predation) affect metacommunity dynamics, causing shifts in spatial and temporal patterns of variation (Cottenie 2005, Thrush et al. 2013, Datry et al. 2016). The soft-bottom coastal ecosystem, for example, is composed by a mosaic of habitats with distinct complexity and environmental characteristics (Zajac

2008) where connectivity and environmental filtering shape the composition and structure of local communities (Vanschoenwinkel et al. 2007, Thrush et al. 2008, Gascón et al. 2016). In these dynamic environments, short-term variability may overcome the variations among seasons and years (Morrisey et al. 1992, Ysebaert and Herman 2002). Nevertheless, we still ignoring this fact when large-term temporal patterns are investigated, jeopardizing our understanding of the processes and mechanisms driven community assembly and dynamics.

The causes of short-term variation in communities are related with environmental and biological factors. Variations in tidal regime, emersion period, salinity, radiation, temperature, sediment texture, and food can operate at multiple temporal scales (Hampel et al. 2003, Tolhurst and Chapman 2005). Species births, deaths, and migration/dispersion also operate at short-time scales, as well interspecific interactions, such as competition, predation, and their resultant behavioral effects, e.g., escape, defense or refuge (Cottenie 2005, Defeo and McLachlan 2005, Zajac 2008). In addition, direct and indirect effects of engineering and bulldozing activities contribute to variation in the structure of benthic communities at short time-scales (Braeckman et al. 2010, 2011, Lohrer et al. 2013). Therefore, the quantitative description of variation in community structure over different temporal scales give us instruments to infer their magnitude and importance, avoiding thus pseudoreplication and confounding effects when long-term events are investigated (Morrisey et al. 1992).

Temporal variation of soft-sediment habitats may have hidden differences. Estuarine tidal flats and sand beaches, for example, have different hydrodynamic regimes and are subjected to distinct environmental fluctuations. Gradients in salinity, sediment texture, and biogeochemical properties are important drivers of environmental filtering at estuarine regions (chapter 2, Valanko et al. 2015). Changes in pluviosity, river run-offs and tides can modify these physicochemical and geomorphological gradients at short and long time-scales affecting soft-bottom communities (Hampel et al. 2003, Tolhurst and Chapman 2005, Pratt et al. 2014). Variability among days and weeks in the tidal heights, temperature and salinity can affect species distribution and community structure (Hampel et al. 2003, Brustolin et al. 2013). Also, seasonal increases in primary production and microbial mat might boost species density and diversity (Franco et al. 2007, 2008, Braeckman et al. 2015).

Sandy beaches on the other hand, are highly dynamic environments shaped by advection energy and geomorphological processes (Defeo and McLachlan 2005, Gheskiere et al. 2005). Seasonal variations in wave and swash regimes affect beach morphodynamics and controls community assembly and dynamics (Defeo and McLachlan 2005). Also, morphodynamics of subtropical sandy beaches can be influenced by meteorological cold fronts, which have a duration of weeks and are more frequent during cold/dry periods, increasing hydrodynamism and sediment disturbance (Gallucci and Netto 2004, Nieto-Ferreira et al. 2011).

Based on this previous information, we believe that sedimentary coastal habitats have their own distinct temporal dynamic. Hence, to evaluate the relationship between temporal variation in sediment properties and ecological communities, we survey free-living nematodes in two different coastal habitats (estuarine tidal flats and sandy beaches) over four temporal scales (seasons, months, weeks, and days) at the subtropical region. Our hypotheses are: If temporal variations in community structure are linked with the prevailing environmental gradients, then estuarine tidal flats and sandy beaches will harbor communities with distinct composition and temporal dynamics. Estuarine tidal flats will be more variable among days and seasons, associated with biogeochemical and food gradients. Conversely, sandy beach will be more variable among weeks, and posteriorly among seasons due to variations associated with cold fronts climatology at the subtropical region. Furthermore, variation in community structure over time (turnover) will be higher in the more dynamic sandy beach environment.

3.2 METHODS

3.2.1 Sampling design

Our study was carried out in Boracéia, at the subtropical coast of São Paulo, Brazil (23°45'34.09" S and 45°53'47.79" O). One site located at an unvegetated tidal flat in the Guaratuba estuary and one site at the adjacent sandy beach were randomly sampled using a nested temporal design (Fig. 1). Habitats were sampled at two main seasons, i.e., colder/dry (June-August) and warmer/wet (December-February). Within each season, two months were randomly chosen. In each of the

four months, two different weeks were selected, and at each week, two days were randomly picked.

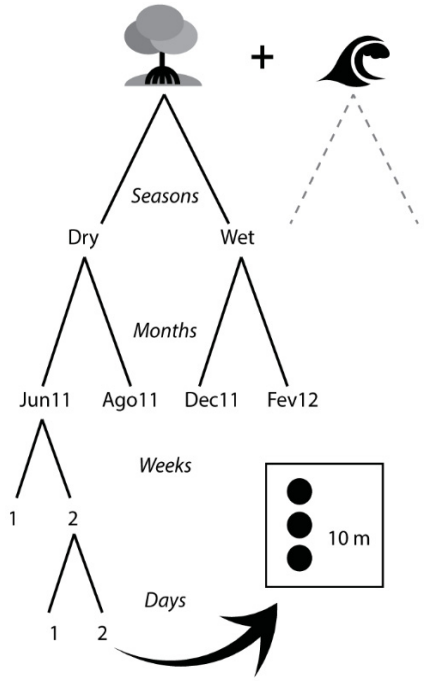

Fig. 1 Sampling design of nematode community across different habitats and time periods.

3.2.2 Sampling and sample processing

At each selected day, three sediment samples were taken in both habitats for meiofauna quantification with a 2.5 cm diameter core, down to a depth of 5 cm. Three other sediment samples (2 g each) were collected for chloroplastic pigment content and granulometric analyses (10 g each). The redox potential was measured at the sediment surface (ca. 1 cm depth) of each sample using a Hanna Instruments HI 991003. Samples for density and species composition analyses were fixed in 4% formaldehyde. Sediment samples for the photosynthetic pigments and granulometric analyses were stored in a cooler in the field and frozen at −20 °C in the laboratory.

Samples taken for the evaluation of density and composition of meiofaunal groups and nematodes were washed through a sieve with a mesh of 45 μm. After washing, the flotation method with colloidal silica solution (Ludox TM-50) adjusted to a specific gravity of 1.18 was used to separate organisms from the sediment (Heip et

al. 1985). All nematodes were counted under a stereoscopic microscope. From each sample, 10 % of the organisms were separated to be mounted in permanent slides. Nematodes were identified under an optical microscope at the lowest possible taxonomic level, by using the identification keys of Platt and Warwick (1983, 1988), Warwick et al. (1998) and the NeMys database (Deprez et al. 2005).

Pigments were extracted with 10 ml acetone (100%) for 24 h in the dark at 4 °C. The extract was centrifuged at 4000 rpm for 5 min. Pigments were analyzed from absorbance spectra at 665 nm and 750 nm before and after acidification with HCL through a digital spectrophotometer. Concentrations of pigments were estimated using the equations of Lorenzen (1967). Sediment granulometry was determined by sieving dried samples and total organic content by difference in dry weight after combustion for 4 hours at 550°C.

3.2.3 Statistical analyses

To visualize the temporal dynamics of communities, non-metric multidimensional scaling (n-MDS) ordinations based on Bray–Curtis dissimilarities of nematode fauna were carried out. Hierarchical permutational multivariate analyses of variance PERMANOVA were used to evaluate the magnitude and significance of variability in nematode fauna among habitats, and time periods (Fig 1). Variation among dry/cold and wet/warm seasons, as well as among the subsequent nested temporal scales (i.e., among months, weeks, and days) were assessed.

Homogeneity of multivariate dispersions PERMDISP (Anderson et al. 2006) were used to test for significant differences in nematode beta diversity among habitats. Constrained analyses of principal coordinates (CAP) were performed to identify possible correlations between temporal dynamics of nematode communities and environmental variables (Anderson and Willis 2003). Sediment grain size fractions, organic matter, chlorophyll-*a* and phaeopigments were included as environmental descriptors. Environmental variables were Hellinger-transformed. ANOVAs were performed to evaluate the significance of the canonical axes. Analyses were conducted in the software PRIMER 6 with PERMANOVA+ add-on (Clarke and Gorley 2006, Anderson et al. 2008) and in the R environment (R Core Team 2017) using the package ‘vegan’ (Oksanen et al. 2016).

3.3 RESULTS

There were significant differences in nematode communities at the estuarine tidal flats and sand beaches. These differences were mainly associated to variations in the composition and temporal turnover of nematode species, whereas richness and total nematode densities did not differ among habitats (see supplementary material, Table S1 and Fig S1). Habitats accounted for 56% of the variation in community structure (Table 1). Differences in seasons and days share the same relative importance (~7%). Species turnover was significant higher in sand beaches compared with turnover at tidal flats as observed by the distance to centroids analysis (F= 4.682 p(perm) = 0.041), and temporal variability in nematode community was lower in the tidal flats, when compared to sandy beaches (Fig. 1a and Fig. S1c., f.).

Communities at sandy beaches were mainly associated with well sorted sediments, with higher percentages of fine and medium sands, and low organic matter content (Fig. 1b). In this habitat, *Daptonema* sp.4 with 26.63 ± 22.31 % (mean ± SD), *Leptogastrella* sp.1 (12.88 ± 16.94 %), *Omicronema* sp.1 (11.90 ± 10.07 %), *Nudora* sp. (6.82 ± 5.03 %), *Pseudosteineria* sp.2 (6.26 ± 5.70 %), and *Oncholaimellus* sp.1 (4.51 ± 5.48 %) were the most abundant nematode species (Fig. 1c).

In tidal flats, nematode community was associated to poorly sorted sediments with higher chlorophyll-*a* and organic matter contents (Fig. 1b). *Sabatieria* sp.1 (27.03 ± 16.48 %), *Cobbia* sp.1 (18.51 ± 15.18 %), *Daptonema* sp.5 (8.23 ± 8.60 %), *Pomponema* sp.1 (6.93 ± 4.34 %), *Neochromadora bonita* (6.65 ± 9.41 %), *Desmodora* sp.1 (6.12 ± 6.83 %), *Odontophora* sp.1 (3.85 ± 3.42 %), and *Cyartonema* sp.1 (1.68 ± 1.65 %) were the dominant species in mudflats. The first two canonical axes together explained 30.4% of the total spatio-temporal variability in nematode fauna (Fig. 1c).

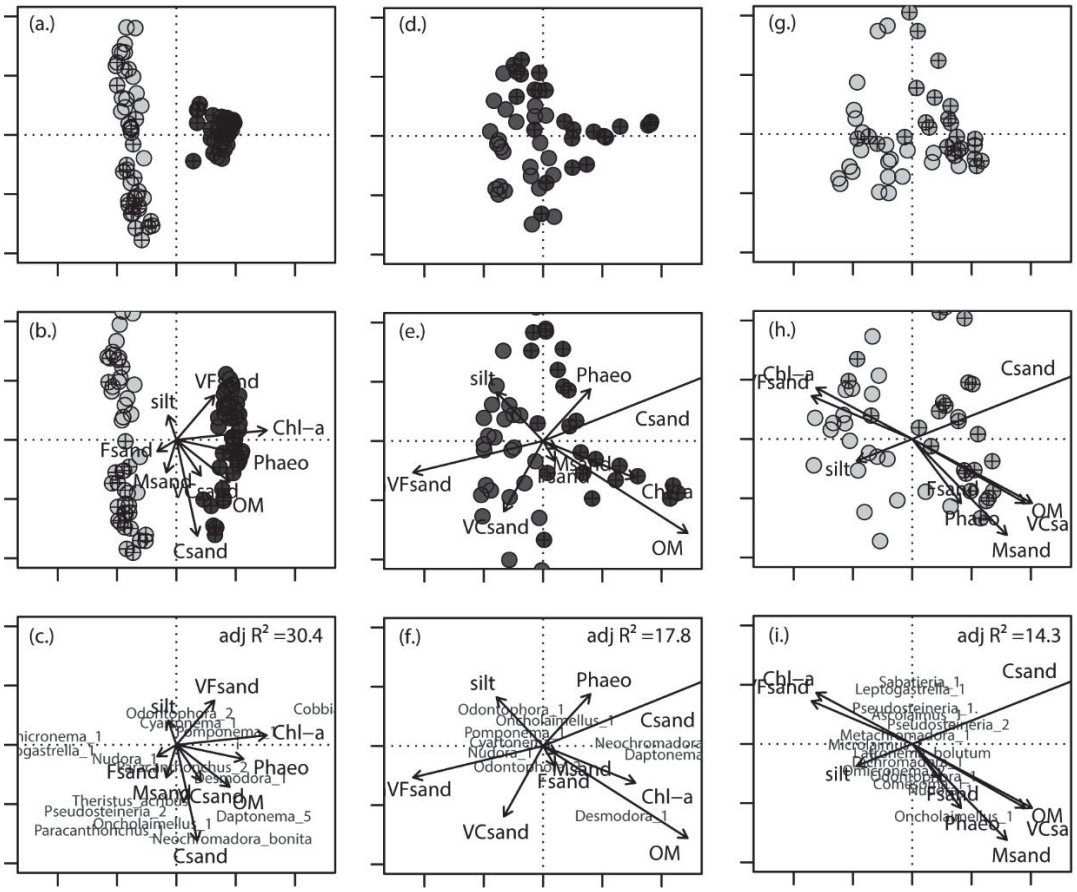

Fig. 2 Unconstrained n-MDS and constrained analysis of principal coordinates (CAP) of the Bray-Curtis dissimilarities on nematode community at estuarine mudflats and sandy beaches across different time periods. Wet season is shown as crossed circles and dry season as open circles. Habitats are shown in different colors (mudflat in dark gray, sand beach in light gray). Ordinations of the overall nematode community (a., b., c.) and of nematode communities at estuarine mudflat (d., e., f.) and sandy beach (g., h., i.). Unconstrained ordinations (a., d., g.). CAP biplots showing habitats and time periods (b., e., h.). CAP biplots showing species' eigenvalues (b., e., h.). Arrows length represent the importance of environmental variables for temporal dynamics of nematode communities.

Looking at the community structure within each habitat separately, we observed that the relative importance of temporal scales differed. The scale of days was the most important source of variation for estuarine fauna, while variation among weeks are more important in sandy beach. In estuarine areas variation within days was significant higher (pseudo-F= 3.726, p(perm)=0.001) and days accounted with 32.3% of the total variability in nematode fauna (Table 1). Variation among months and seasons were less important and together comprised about one third of total variability in nematode community (Table 1). Nematode fauna was associated to higher contents of coarse sand, organic matter, and chlorophyll-*a* at warm/wet season (Fig. 1e). *Sabatieria* sp.1, *Daptonema* sp.5, *Cobbia* sp.1, *Desmodora* sp.1, and *Neochromadora bonita* were the most representative taxa in wet periods, while

Pomponema sp.1, *Odontophora* sp.1, and *Cyartonema* sp.1 were more abundant in dry periods (Fig. 1f). The two canonical axes together explained 17.8% of the total temporal variability in nematode fauna (Fig. 1f).

In sandy beach, variation among weeks was significant (pseudo-F= 2.100, p(perm)=0.023), accounting with 13.98 % of the total variability in nematode fauna. Also, variation between seasons comprised 18.45 % of the total nematode variability (Table 1). In wet periods nematode fauna were mainly associated with higher percentages of coarse sand and organic matter content (Fig. 1g, h, i). Canonical axes together explained 14.3% of the total temporal variability in nematode fauna (Fig. 1i)

Table 1 Permutational multivariate analyses of variance PERMANOVA for temporal dynamics of nematode communities at overall system, Unvegetated tidal flat and Beach. Analyses were based on Bray-Curtis dissimilarity matrices. Bold letters highlight terms with significant effects ($p < 0.005$), df = degrees of freedom, MS = mean squares.

	Overall nematode community				
	df	MS	Pseudo-F	P(MC)	CV (%)
Habitat	1	1.31^{E+05}	10.856	0.004	57.89
Seasons	2	12069	2.745	0.004	7.46
Months	4	4396	1.678	0.021	3.46
Weeks	8	2619	1.416	0.036	2.99
Days	16	1848	2.087	0.001	7.49
Res	64	885	-	-	20.68
Total	95	-	-	-	-
	Estuarine tidal flat				
	df	MS	Pseudo-F	P(MC)	CV (%)
Seasons	1	11891	2.370	0.094	17.37
Months	2	5016	2.206	0.052	13.86
Weeks	4	2273	1.041	0.436	0.91
Days	8	2183	3.726	**0.001**	32.30
Res	32	586	-	-	35.54
Total	47	-	-	-	-
	Sandy Beach				
	df	MS	Pseudo-F	P(MC)	CV (%)
Seasons	1	12393	3.157	0.057	18.45
Months	2	3924	1.281	0.298	3.75
Weeks	4	3062	2.100	**0.023**	13.98
Days	8	1458	1.324	0.086	6.22
Res	32	1101	-	-	57.58
Total	47	-	-	-	-

3.4 DISCUSSION

Habitats hosted communities with distinct structure, composition and temporal dynamics. Environmental filtering drives metacommunity assembly in soft-sediments. The balance between physical/advection and biogeochemical/diffusion gradients is the major structuring force at the costal landscape (Chapter 2, Gascón et al. 2016). In the low advection estuarine tidal flats, nematodes were associated to poorly sorted sediments with high chlorophyll-*a* and phaeopigment contents. These proxies of fresh microphytobenthic production and phytodetritus contents were highly variable at short time-scales (Fig. S2). In estuarine environments, daily variations in tidal amplitude, emersion, irradiation, and salinity may modify sediment geochemistry and food availability (Tolhurst and Chapman 2005, Maggi et al. 2012), affecting thus the temporal variation patterns of soft-bottom estuarine community (Steyaert et al. 2001, Hampel et al. 2003, Brustolin et al. 2013). Conversely, nematode community in sand beaches were more influenced by the balance between sediment grain size fractions, at short and longer time scales (Figs. S3, S4). Therefore, physical/geomorphological processes are the main drivers of community structure (Gheskiere and Vincx 2004, Gheskiere et al. 2005), and the degree of advection (e.g., exposure to wave action) is determinant for temporal variation in nematode fauna (Gallucci and Netto 2004, Gheskiere and Vincx 2004). Hence, nematode metacommunities from coastal ecosystem were affected by a combination of factors acting at multiple spatio-temporal scales.

Short-term temporal variations in community structure and composition were equal or even more important than variation among months and seasons. A similar pattern has already been observed for macrobenthic and meiobenthic communities (Morrisey et al. 1992, Ysebaert and Herman 2002, Azovsky et al. 2004). The high variability in community structure found within and among days suggest temporal dynamics of meiofauna is driven not only by long-term seasonal changes and large-scale oceanographic processes such, waves and tidal currents, but also by factors acting at small spatial and temporal scales (Morrisey et al. 1992, Tolhurst and Chapman 2005). Such factors might include localized water movements and their associated variables, such as food supply, oxygen, pollutants (Blanchard 1990, Chapman et al. 2010, Souza et al. 2016). Biotic factors such as competition, predation, engineering, and bulldozing may also play an important role in small-scale

variability (Gallucci and Netto 2004, Braeckman et al. 2011, Lohrer et al. 2013). Moreover, nematode's life cycle and colonization/succession dynamics operate in shorter periods of time (Van Colen et al. 2009), which might increase short-term temporal variability. Hence, the magnitude of small-scale variability cannot be ignored in studies of benthic temporal dynamics. Otherwise, seasonal effects can be confounded with short-term changes masking the real patterns of temporal variation in communities.

Despite being lower, variation among seasons was also important. These variations may be related with changes in physicochemical gradients at estuarine regions, while in sandy beaches changes in advection intensity and geomorphology along the year affect community dynamics. For example, changes in pluviosity, primary productivity, and oxygen availability can affect the seasonal patterns of nematode fauna (Vanaverbeke et al. 2004, Fonseca and Netto 2006, Van Colen et al. 2009). In warm/rain periods, mud and nutrients runoffs are higher which increases primary production and the microbial mat in coastal and estuarine regions (Fonseca and Netto 2006, Franco et al. 2007). Consequently, respiration also increases, favoring deposit and epistrate feeders tolerant to hypoxia such as *Sabatieria* and *Desmodora* (Franco et al. 2008, Braeckman et al. 2015). Therefore, seasonal dynamics of estuarine nematode communities must be driven by trade-offs between food and oxygen availability.

In subtropical sand beaches conversely, variability in nematode fauna is mainly associated with the occurrence of meteorological cold fronts (Gallucci and Netto 2004) which generally have a stationary period of weeks (Nieto-Ferreira et al. 2011). The frequency as well the intensity of frontal systems are generally higher during cold/dry periods increasing hydrodynamism and sediment disturbance (Gallucci and Netto 2004, Nieto-Ferreira et al. 2011). Consequently, weeks and seasons were the main sources of variation in nematode community at exposed sand beach habitat. In cold/dry season, when erosion and disturbance events are more frequent (Defeo and McLachlan 2005), sediments were composed of high percentages of well-sorted very fine sands (Fig. S4). While, sediment sorting was lower, and percentages of fine and coarse sands were higher in warm/wet periods when events of sediment accretion in beach profile are more frequent (Defeo and McLachlan 2005). This suggests that hydrodynamic energy act over multiple

temporal scales and can drive either short-term variability or seasonal dynamics of nematode communities at sand beaches.

Variation in community structure was higher at more hydrodynamic sandy beaches if compared with more protected estuarine tidal flats. This might have relation with differences in nematode dispersal/connectivity among habitats (see chapter 2). The advection energy is higher at sand beaches, waves and currents are constantly remobilizing and redistributing sediments increasing passive dispersal, preventing local extinctions, and reducing the role of environmental filtering in a mass-effect dynamic (chapter 2, Cottenie 2005). Conversely in estuarine tidal flats, advection intensity is lower and environmental gradients in sediment biogeochemistry and food availability were the main structuring forces (chapter 2). Hence, local communities were more stable through time and variation in community structure is driven by environmental filtering in a more species-sorting or patch-dynamics (chapter 2, Gascón et al. 2016). Understand the complex interplay between ecological and environmental processes affecting the structure and temporal variation of local communities in heterogeneous landscapes such as marine soft-bottoms is thus essential to increase our knowledge about mechanisms controlling metacommunity organization and dynamics (Thrush et al. 2008, 2013).

Based on our results we conclude that habitats act as environmental filtering hosting communities with different temporal dynamics. Short-term temporal variability has equal or more importance than seasonal variations for the community structure of small invertebrates. Sand beaches are more variable, and dispersal/connectivity prevents local species extinction increasing turnover in space and time. Our results show the importance of temporal replication at shorter time scales, when long-term dynamics are studied. These highlights the need to evaluate variability over multiple temporal scales to fully understand the effects of environmental and ecological processes on the structure and dynamics of benthic communities.

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SUPPLEMENTARY MATERIAL

Figures

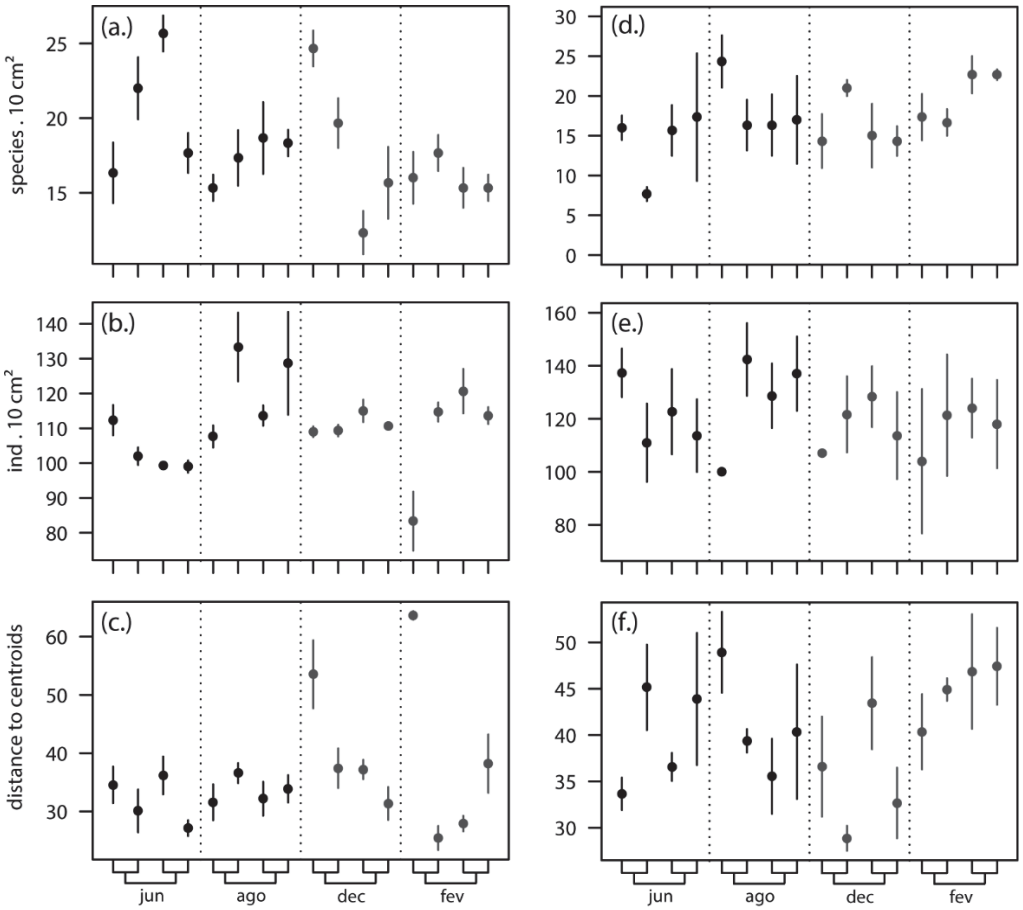

Fig. S1 Temporal variation in species richness, total density, and distance to centroids of nematode fauna from estuarine tidal flat (a., b., c.) and sand beach habitats (d., e., f.). Points are sampling days (mean ± SE, n=3). Cold/dry season are shown as black dots and warm/wet period as gray dots.

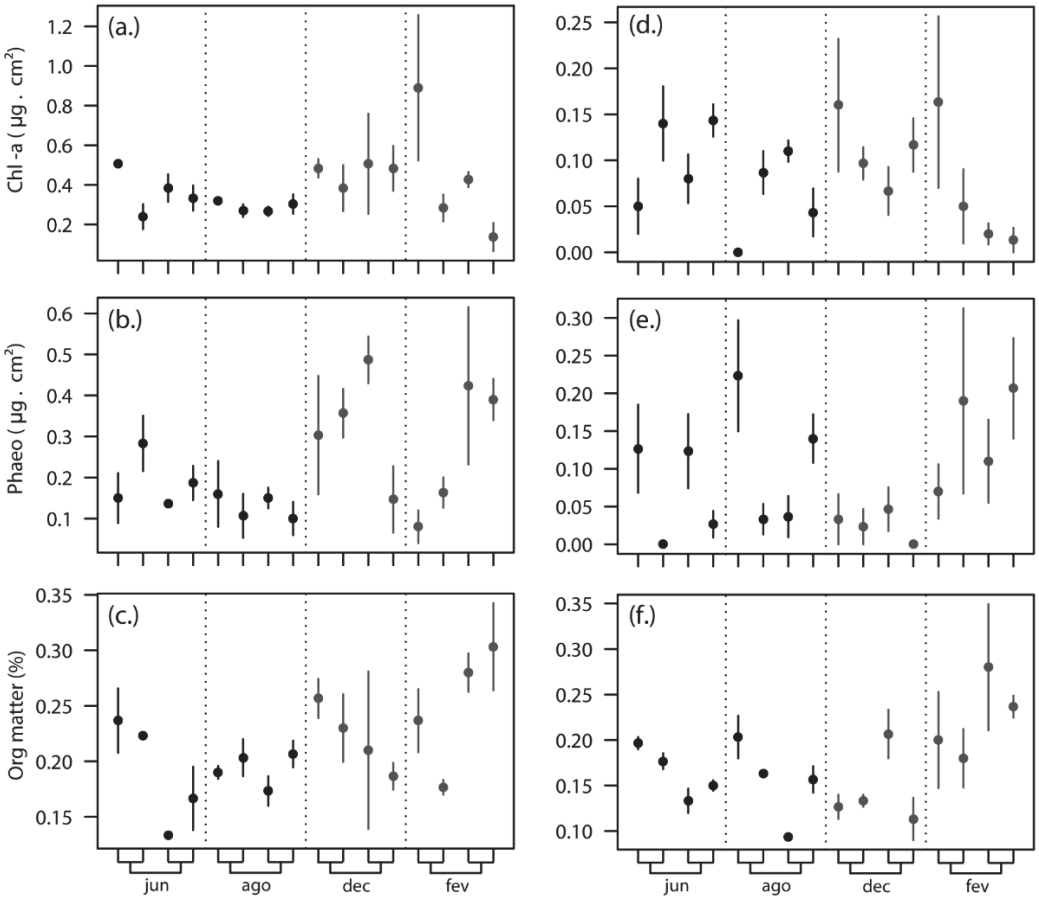

Fig. S2 Temporal variation in chlorophyll-*a*, phaeopigments, and organic matter contents from estuarine tidal flat (a., b., c.) and sand beach sediments (d., e., f.). Points are sampling days (mean ± SE, n=3). Cold/dry season are shown as black dots and warm/wet period as gray dots.

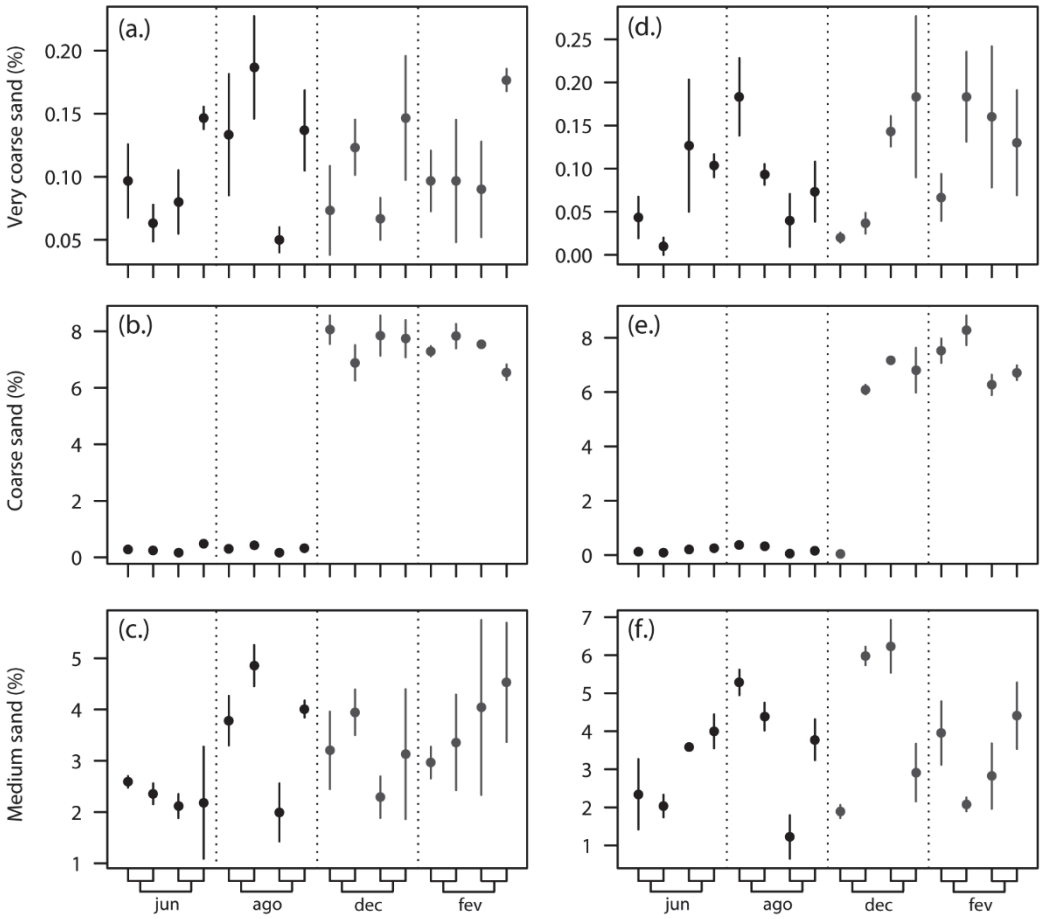

Fig. S3 Temporal variation in the percentages of very coarse, coarse, and medium sands at estuarine tidal flat (a., b., c.) and sand beach sediments (d., e., f.). Points are sampling days (mean ± SE, n=3). Cold/dry season are shown as black dots and warm/wet period as gray dots.

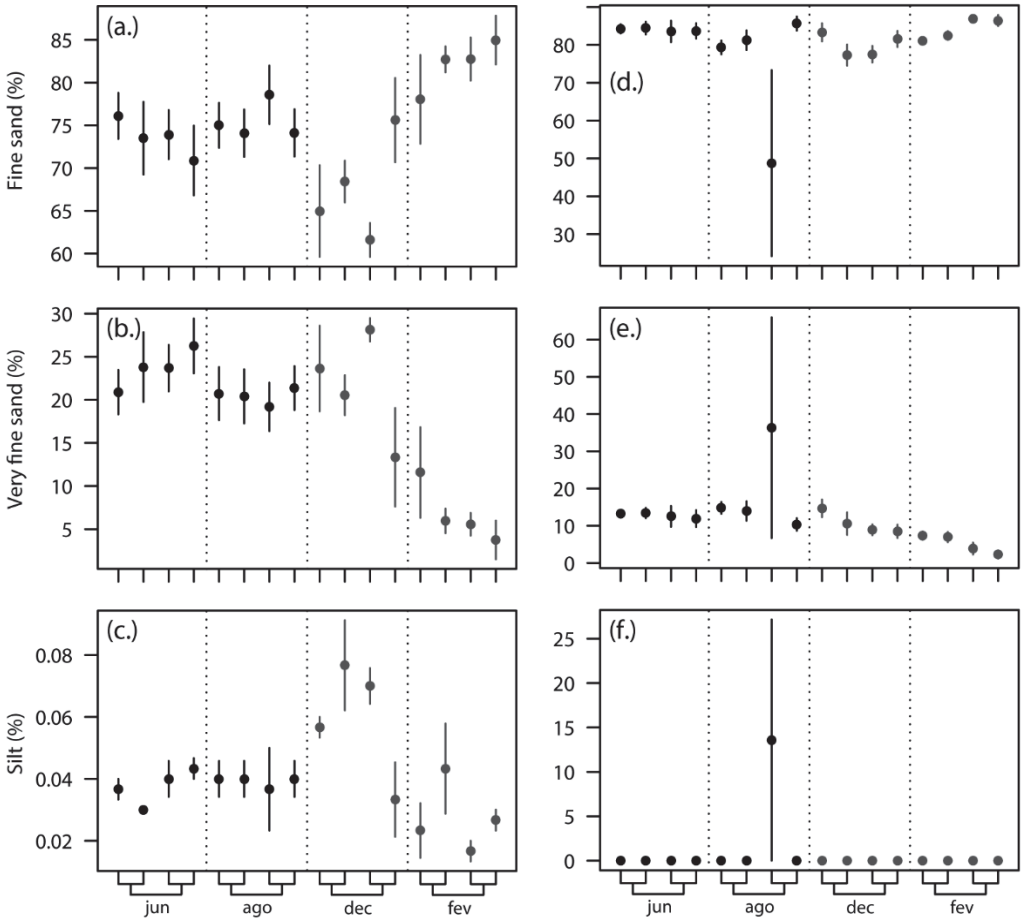

Fig. S4 Temporal variation in the percentages of fine sands, very fine sands, and silt at estuarine tidal flat (a., b., c.) and sand beach sediments (d., e., f.). Points are sampling days (mean ± SE, n=3). Cold/dry season are shown as black dots and warm/wet period as gray dots.

Tables

Table S1 PERMANOVA table of results for temporal dynamics in nematode richness, and total densities for communities at overall system, unvegetated tidal flat and sandy beach. Analyses were based on Euclidean distances.
df = degrees of freedom, MS = mean squares.

	Overall species richness					Overall total density			
	df	MS	Pseudo-F	P(MC)	CV (%)	MS	Pseudo-F	P(MC)	CV (%)
Habitat	1	16.66	0.671	0.574	0	2350.30	7.427	0.111	9.68
Seasons	2	36.83	0.640	0.625	0	316.43	0.593	0.609	0
Months	4	67.83	0.412	0.395	2.70	533.01	2.048	0.176	5.19
Weeks	8	58.58	0.103	0.129	16.67	260.22	0.509	0.831	0
Days	16	30.10	0.179	0.182	9.57	510.28	1.344	0.196	9.97
Res	64	21.92	-		71.06	379.41			75.16
Total	95	-	-	-	-	-	-	-	-
	Tidal flat species richness					Tidal flat total density			
	df	MS	Pseudo-F	P(MC)	CV (%)	MS	Pseudo-F	P(MC)	CV (%)
Seasons	1	40.33	1.034	0.418	2.99	72.52	0.075	0.811	0
Months	2	39.00	0.650	0.565	0	961.85	3.144	0.143	27.47
Weeks	4	59.91	2.297	0.16	30.16	305.94	0.799	0.548	0
Days	8	26.08	3.347	0.007	31.37	382.56	4.048	0.003	36.40
Res	32	7.79			35.46	94.50			36.12
Total	47	-	-	-	-				
	Sandy beach species richness					Sandy beach total density			
	df	MS	Pseudo-F	P(MC)	CV (%)	MS	Pseudo-F	P(MC)	CV (%)
Seasons	1	33.33	0.344	0.601	0	560.33	5.379	0.154	14.46
Months	2	96.66	1.688	0.271	18.53	104.17	0.485	0.666	0
Weeks	4	57.25	1.677	0.256	20.07	214.50	0.336	0.84	0
Days	8	34.12	0.946	0.503	0	638.00	0.960	0.467	0
Res	32	36.06			61.39	664.31	-	-	85.53
Total	47	-	-	-	-	-	-	-	-

Table S2 Average densities and relative abundances (mean ± SD) of nematode species among seasons at each habitat. dens = density (ind. 10 cm^2), ab = relative abundance (%), and sd= standard deviation.

	Sandy Beach								Estuarine tidal flat							
	Cool/dry				Warm/wet				Cool/dry				Warm/wet			
	dens	sd	ab	sd	dens	sd	ab	sd	dens	sd	ab	sd	dens	sd	ab	sd
Actionema 1	0.2	0.6	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aerolaimus 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Aerolaimus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Anonchus laureata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.2	0.4	0.2	0.4
Anoplostoma 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Anoplostoma 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Ascolaimus 1	1.9	2.2	1.4	1.6	3.2	2.5	3.0	3.2	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.3
Ascolaimus acribus	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Atholaimus 1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bathylaimus 1	0.3	0.6	0.2	0.4	0.8	1.8	0.8	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bathylaimus 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.1	0.4	0.0	0.0	0.0	0.0
Bolbolaimus 1	1.3	3.2	1.2	3.3	0.2	0.6	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Camacolaimus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Catanema 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ceramonema 1	0.0	0.0	0.0	0.0	0.1	0.4	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chromadorita 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0
Chromadorita 1	0.1	0.3	0.1	0.2	0.2	0.6	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chromaspirinia 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Chronogaster alatum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.3
Cobbia 1	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	21.7	11.5	19.4	10.3	20.3	22.2	17.7	19.1
Cobbia 5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Coinkia 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.2
Comesoma 1	1.2	2.7	1.0	2.5	1.3	2.1	1.1	1.7	0.3	0.5	0.2	0.5	0.2	0.4	0.2	0.4
Conilia divina	0.7	1.3	0.6	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyartonema 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	1.9	2.4	2.0	1.0	0.9	0.9	0.8
Cyartonema 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Cyartonema 3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Daptonema 1	0.2	0.5	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0
Daptonema 3	0.5	1.1	0.4	1.0	0.1	0.4	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Daptonema 4	46.3	21.5	39.3	22.1	15.0	13.7	13.9	13.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Daptonema 5	0.0	0.0	0.0	0.0	0.3	0.6	0.2	0.6	4.3	2.8	3.9	2.8	13.2	10.8	12.5	10.3
Dasynemella 2	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.3	0.7	0.3	0.7	0.3	0.7	0.2	0.7
Dasynemella cincta	0.2	0.5	0.1	0.4	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dasynemoides 1	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Desmodora 1	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	4.3	4.8	4.0	4.3	8.5	7.9	8.3	8.2
Dichromadora 1	0.5	1.6	0.5	1.6	0.1	0.3	0.1	0.2	0.3	0.8	0.2	0.6	0.1	0.3	0.1	0.3
Dichromadora 2	0.1	0.3	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Dichromadora 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.5	0.2	0.5	0.1	0.3	0.1	0.3
Enoploides 1	0.1	0.4	0.1	0.3	0.3	1.1	0.3	1.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Eubostrichus 1	0.0	0.2	0.0	0.2	0.2	0.4	0.2	0.4	0.0	0.2	0.0	0.2	0.5	0.8	0.4	0.7
Eurystomina 1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eurystomina 2	0.1	0.4	0.1	0.3	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eurystomina sawayai	0.3	1.2	0.2	0.9	0.2	0.4	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nematode spp 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Nematode spp 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Gomphionema 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.6	1.4	1.5	1.8	6.1	1.7	5.5
Gonionchus 1	0.8	1.4	0.7	1.3	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gonionchus 2	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Halalaimus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.2	0.4	0.0	0.0	0.0	0.0
Halalaimus 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Halichoanolaimus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2
Hipodontolaimus 1	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.3	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Hypodontolaimus 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Indet	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.0	0.4	1.0	0.5	0.9	0.5	0.9
Latronema 1	0.3	0.9	0.2	0.6	0.3	0.6	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Latronema botulum	0.7	1.8	0.6	1.7	1.1	2.3	0.9	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lauratonema 1	0.1	0.4	0.1	0.3	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lauratonema hospitum	0.1	0.4	0.1	0.3	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Leptogastrella 1	16.6	27.5	12.9	19.6	15.3	16.9	12.9	14.3	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Leptogastrella 2	0.0	0.0	0.0	0.0	0.1	0.4	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Linhomoeus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Metachromadora 1	0.8	1.3	0.6	0.9	0.5	0.8	0.4	0.9	0.1	0.3	0.1	0.3	0.0	0.2	0.0	0.2
Metachromadora 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0
Metadasynemoides 1	0.0	0.2	0.0	0.2	0.1	0.3	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Metadasynemoides lata	0.3	1.0	0.2	0.7	0.7	1.1	0.6	1.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2

Metalinhomoeus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Meylia 1	0.5	0.8	0.3	0.5	0.8	1.1	0.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microlaimus 1	0.4	1.0	0.4	0.9	0.0	0.0	0.0	0.0	0.5	1.1	0.4	0.9	0.1	0.3	0.1	0.3
Microlaimus 3	0.2	0.8	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microlaimus 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0
Microlaimus 5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.1	0.3	0.1	0.3
Microlaimus 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.2	0.4	0.0	0.2	0.0	0.2
Microlaimus 7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.2
Microlaimus formosus	1.8	2.4	1.4	2.0	0.8	2.1	0.6	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microlaimus indet	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microlaimus papilatus	0.0	0.0	0.0	0.0	0.5	0.8	0.4	0.8	0.1	0.3	0.1	0.3	0.2	0.8	0.2	0.8
Monhystrella 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Nannolaimoides 1	0.1	0.4	0.1	0.3	0.3	0.8	0.2	0.6	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0
Neochromadora bonita	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.8	1.4	1.8	12.1	9.7	11.9	11.0
Nudora 1	7.3	7.8	5.6	5.3	9.5	5.5	8.1	4.5	4.0	3.2	3.6	2.8	0.8	1.9	0.8	1.8
Odontophora 1	0.5	1.2	0.3	0.8	1.7	4.5	1.2	3.0	5.2	3.9	4.6	3.4	3.5	3.8	3.1	3.4
Odontophora 2	0.5	1.6	0.4	1.1	0.2	0.5	0.2	0.5	1.7	2.3	1.5	2.2	0.2	0.4	0.2	0.4
Omicronema 1	11.9	10.6	9.5	7.9	15.8	12.6	14.3	11.6	0.3	0.6	0.3	0.6	0.0	0.0	0.0	0.0
Omicronema 2	1.0	2.5	0.9	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Omicronema 4	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oncholaimellus 1	3.8	5.0	3.0	3.6	7.5	8.5	6.1	6.6	1.3	1.9	1.1	1.6	2.8	5.6	2.5	5.1
Oncholaimus 1	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.4	0.8	0.4	0.8	0.2	0.6	0.1	0.6
Oxystomina 1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.9	1.0	0.8	0.9	0.3	0.7	0.3	0.6
Oxystomina 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.2	0.2	0.4	0.2	0.4
Oxystomina 5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Paracanthonchus 1	6.6	12.2	4.6	7.9	10.8	9.8	8.7	7.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paracanthonchus 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.8	0.6	0.8	2.5	4.9	2.3	4.2
Paracomesoma 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3
Paracyatholaimoides 1	0.1	0.3	0.1	0.3	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.8	0.2	0.7
Paralinhomoeus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
Paralongicyatholaimus 1	0.4	1.3	0.4	1.3	1.5	4.1	1.2	2.8	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Paramonhystera 1	0.3	0.7	0.3	0.7	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.7	1.5	0.6	1.4
Polygastrophora 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.3	0.5	0.3	0.5
Pomponema 1	0.5	1.0	0.4	0.8	0.3	0.8	0.3	0.6	8.0	4.6	7.5	4.8	7.2	4.5	6.4	3.9
Pontonema 1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	2.9	2.8	2.5	2.5	1.6	2.8	1.4	2.5

Prorchynchonema 1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0
Pselionema 2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudosteineria 1	0.1	0.3	0.1	0.2	1.3	2.9	1.1	2.2	0.2	0.4	0.1	0.3	0.0	0.2	0.0	0.2
Pseudosteineria 2	5.5	6.8	4.2	5.1	10.1	7.0	8.3	5.7	0.0	0.2	0.0	0.1	0.1	0.4	0.1	0.4
Rhips 1	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhynchonema 1	0.3	0.8	0.3	0.5	0.5	1.0	0.5	0.9	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Sabatieria 1	3.1	5.3	2.2	3.7	4.5	3.5	3.7	2.9	40.1	21.5	34.6	15.0	22.1	16.5	19.4	14.5
Sabatieria 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.1	0.4
Sabatieria 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Scaptrella 1	0.0	0.2	0.0	0.1	0.0	0.2	0.1	0.3	1.9	1.9	1.7	1.8	1.0	1.7	0.8	1.5
Scaptrella 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Southerniella 1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Sphaerolaimus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Sphaerotheristus 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.2	0.4	1.8	2.3	1.7	2.1
Sphaerotheristus 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Spirinia 1	0.0	0.2	0.0	0.1	0.0	0.2	0.0	0.1	0.3	0.5	0.2	0.5	0.1	0.4	0.1	0.4
Spirinia 2	0.1	0.4	0.1	0.4	0.6	1.7	0.5	1.3	0.2	0.4	0.2	0.4	0.8	1.1	0.7	1.0
Spirinia 3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3	0.3	0.7	0.3	0.6
Stylotheristus 1	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.9	1.4	0.8	1.3	1.4	1.9	1.2	1.7
Synonchiella 1	0.0	0.0	0.0	0.0	0.4	1.0	0.3	0.8	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3
Synonchiella 2	0.1	0.6	0.1	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Terschillingia 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Theristus 1	0.2	0.6	0.1	0.4	0.5	0.7	0.4	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Theristus 5	0.1	0.4	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Theristus acribus	2.8	3.1	2.1	2.1	7.3	8.0	5.8	5.4	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.3
Theristus flevensis	0.1	0.3	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.2	0.1	0.3
Theristus sawaya	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Theristus stranus	0.2	0.5	0.1	0.4	0.6	1.2	0.5	0.8	0.0	0.2	0.0	0.1	0.1	0.3	0.1	0.3
Trefusia 1	0.1	0.3	0.1	0.2	0.0	0.0	0.0	0.0	1.4	2.1	1.3	2.1	0.1	0.4	0.1	0.3
Trefusia 2	0.0	0.2	0.0	0.2	0.2	0.5	0.1	0.4	0.0	0.0	0.0	0.0	0.1	0.4	0.1	0.4
Trefusia acribus	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trileptium 1	1.0	2.0	0.7	1.4	0.5	0.7	0.4	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trochamus 1	0.0	0.2	0.0	0.1	0.0	0.2	0.0	0.2	0.4	0.8	0.4	0.8	0.2	0.4	0.2	0.4
Viscosia 1	0.3	0.5	0.2	0.5	0.1	0.3	0.1	0.2	0.2	0.5	0.2	0.5	0.3	0.7	0.2	0.7
Xyala 1	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

4 CHAPTER 4: GLOBAL WARMING AND OCEAN ACIDIFICATION REDUCE DIVERSITY AND SPECIES TURNOVER AMONG MARINE HABITATS

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Abstract

Climate change has been forecast to drive shifts in species compositions and increase the dominance of generalist species. These predictions are largely based on single habitat studies, but in nature, habitats are often ecologically connected through exchange of energy and organisms. Here, we investigated the interactive impacts of ocean warming and acidification on the structure of marine benthic communities from three distinct, but connected shallow-water habitats: sandy soft-bottoms, marine vegetation (artificial seagrasses) and rocky reef substrates. The isolated and combined effects of temperature and acidification were tested using a long-term large mesocosm experiment with meiofauna as a model species group. The combined effect of temperature and acidification was stronger than each factor in isolation, showing an overall increase in net primary production and detritus biomass. The combined climate stressors further drove a homogenization of the

fauna across habitats. Under present-day conditions, metacommunities were structured by variability in food, but under future conditions they showed an unstructured, random pattern. Whilst some nematode species are generally resistant to changes in temperature, and their densities increased under future climates, the functional and trophic diversity of community decreased. The latter was also observed for copepods, which are often more sensitive to environmental changes than nematodes. Elevated CO_2 can hence increase productivity homogenizing food availability among habitats, while warming will exclude sensitive species leading to a facilitation of resistant non-selective deposit feeders. Such biodiversity homogenization could affect ecosystem functioning with consequent cascading effects on marine food-webs.

Keywords: *Beta-diversity; Climate change; Metacommunity; Seascape heterogeneity*

4.1 INTRODUCTION

Rising anthropogenic CO_2 levels and the consequent increase in temperature and acidification of the ocean is impacting biodiversity and will change ecosystems in ways that are still difficult to forecast with great certainty. Understanding the consequences of climate change and mitigating its impacts are urgent priorities for the conservation and management of species and human societies (Doney et al., 2012; Nagelkerken & Connell, 2015). Variation in CO_2 levels and temperature of seawater may affect ecological processes by increasing carbonate solubility (affecting species physiology), altering animal behaviour, changing food availability, reducing predator densities or altering competitive hierarchies (Connell et al., 2017; Doney et al., 2012; Hale et al., 2011). Responses to climate change are often species-, latitude-, context-, and ecosystem-specific (Hale et al., 2011; Nagelkerken & Connell, 2015), but can be exacerbated or buffered by species interactions (Goldenberg et al., 2018). Likewise, climate change effects may be altered by interactions between adjoining habitats, e.g. through energetic fluxes or animal migrations (Doney et al., 2012; Urban et al., 2012). Ecological communities that occur in proximity to one another and are genetically

connected (i.e. metacommunities) share a significant amount of resources and species (Leibold et al., 2004). This provides opportunities for buffering of disturbance effects through ecological connectivity where local community responses may not extend to broader responses at metacommunity levels.

At present, there is still little understanding of how species- and meta-communities are affected by climate change (Prosser et al., 2007; Magurran et al., 2015). Generally, the focus of metacommunity studies is on the processes and mechanisms influencing the patterns of local communities at multiple spatial and temporal scales (Leibold et al., 2004). Recent evidence of climate-driven effects on marine communities suggests ocean warming and acidification might lead to phase-shifts, generally associated with changes in species composition and decreases in diversity (Connell & Russell, 2010; Hillebrand et al., 2010; Meadows et al., 2015; Nagelkerken et al., 2017). Climate change may also cause a decrease in species turnover (i.e., beta-diversity), often referred to as community homogenization (García Molinos et al., 2015). Homogenization is frequently related to range expansions of generalist species, rather than by an overall decrease in diversity (García Molinos et al., 2015; Magurran et al., 2015). The combined effect of community homogenization and biodiversity loss are likely to lead to a decrease in ecosystem functioning and productivity (Doney et al., 2012; Nagelkerken & Connell, 2015; Ramírez et al., 2017). Despite this, there is little information on how climate change can drive homogenization of marine habitats. In terms of metacommunity theory, homogenization may be generated by local extinctions, which remove strong competitors that otherwise would prevent adaptation of nonresident species to new niches (Urban 2012). This support the idea that under climate change, local communities will become more similar, and generalist species will dominate all habitats changing the overall metacommunity structure.

The spatial structure of a metacommunity is best described by the combination of three emerging properties from its species distributions: coherence, turnover, and species range boundaries (Leibold & Mikkelson, 2002; Presley et al., 2010). Through the evaluation of these three metrics, it is possible to reveal metacommunity patterns and infer mechanisms that most likely contribute to metacommunity organization (Gascón et al., 2016). The elements of metacommunity structure (EMS) is a framework where empirical data from metacommunity compositions can be fitted into twelve theoretical spatial distribution

models (Presley et al., 2010). Nested subsets for example, are characterized by richness differences among local communities, while Gleasonian, Clementsian, and evenly-spaced distributions are associated with high species turnover (Gascón et al., 2016). Meta-communities can also can show a random structure, where species respond differently to multiple environmental gradients, or a checkerboard pattern where interspecific competition results in mutual exclusion of species (Leibold & Mikkelson, 2002; Presley et al., 2010). Incorporating a metacommunity framework into climate change studies can provide more accurate predictions about future changes in species distributions and biodiversity loss at broader scales (Urban et al., 2012).

To understand the potential effects of climate change on metacommunity structuring, we conducted a mesocosm experiment with natural meiobenthic communities from three distinct habitats. Meiofauna is composed of small benthic organisms (<0.5 mm), with short generation times (weeks to months) which occur in high densities (one million per square meter; Heip et al., 1985). Moreover, meiofaunal communities are characterized by a high taxonomic and functional diversity comprising nearly all invertebrate animal phyla (Giere, 2009; Zeppilli et al., 2015). All these attributes make them a useful group for manipulative experiments. Here we examined the potential impact of elevated CO_2 and temperature on a meiofauna metacommunity from bare sand, seagrass and rocky reef habitats, using large mesocosms (1,800 L), with long-term (6 months) exposure to climate stressors. In nature, these habitats are known to harbor communities that differ in terms of diversity and dominance of major taxa (Danovaro et al., 2007; Fonseca et al. 2015). We hypothesized that under future warming and acidification scenarios, habitats will suffer from significant community changes and increased species overlap due to a homogenization effect facilitated by increased predominance of a few, but generalist species. Specifically, we tested whether climate change can cause a shift from a metacommunity structured by niche-based dynamics in the distinct habitats, to a random type where spatial homogenization in food availability damp environmental filtering.

4.2 MATERIAL AND METHODS

4.2.1 Experimental set-up

We built a sophisticated long-term mesocosm system designed to simulate a shallow temperate marine ecosystem at the South Australian Research and Development Institute (SARDI). The experiment ran for six months (February 2015 – August 2015) and comprised two 800 L header tanks, 12 1,800 L mesocosm tanks, and 12 60 L enrichment tanks. All tanks were placed inside a controlled temperature room with ambient air temperature set at 23 ºC. All mesocosm tanks were under the same light and nutrient regime. The header tanks were used to pre-treat the water to future climate conditions and were connected to the respective mesocosms (see climate treatments below for a better understanding). Each mesocosm tank was illuminated by an overhanging 250-watt high-pressure metal halide lamp (Osram Powerstar HQI-T 250/D/PRO) for a period of 14 hours per day, simulating the duration of mid-latitude summer daylight (Bureau of Meteorology, www.bom.gov.au, location Adelaide). The light bulbs were selected based on their ability to emit near-natural sunlight (according to the spectrum provided by the manufacturer) and each mesocosm received an irradiance at the bottom of the tank of 3833 ± 1304 lux corresponding to ~10 meters depth in average temperate coastal waters (Phillips, 1981; Sverdrup et al., 1942). Nutrients were supplied by an inflow of unfiltered seawater from an offshore pipeline located 1.5 km from the coast at 8 m depth at a rate of 1.72 ± 0.04 $L.min^{-1}$/tank, with a total of ~1,800 $L.day^{-1}$/tank. Excess water left the mesocosms through a central ~20 µm mesh filter column, where it was collected by gravity in the enrichment tanks. These enrichment tanks were placed next to each mesocosm tank and maintained the respective climate treatments of the water, which was recycled back to the mesocosm with a pump. Each enrichment tank contained two ~1.8 $m^3.h^{-1}$ pumps, and the elevated temperature mesocosms housed two submersible titanium heaters (300 W and 500 W) whilst all mesocosms were heavily aerated with either ambient or CO_2-enriched air. The two pumps that returned the treatment water from the enrichment tanks to the mesocosms were set to alternately the direction of water flow every six hours mimicking a tidal current change (i.e. changing the direction of the water circulation

inside the mesocosm tank) and the excess water was then discarded via gravity through an outflow.

4.2.2 Climate treatments

Our climate treatments were based on IPCC predictions for the year 2100 following Representative Concentration Pathway (RCP) 8.5, which represent a business-as-usual CO_2 emission scenario (IPCC, 2014). The experiment was carried out in a crossed design using current (400 ppm) and future (1,000 ppm) target concentrations of CO_2 in combination with average present (21.5°C) and future (24°C) summer temperatures, with each treatment having three replicate mesocosms (Goldenberg et al., 2017). To achieve the expected future pH scenarios pure CO_2 was first dissolved in the header tanks water before entering the mesocosms. CO_2 levels were then maintained in the both elevated CO_2 treatments by bubbling enriched air into the respective enrichment tanks using a PEGAS 4000 MF Gas Mixer controller (Columbus Instruments, CO, USA) set at 1,000 ppm pCO_2. Bubbling occurred only in the enrichment tanks so as to not disturb or stress the mesocosm community. As representative for natural systems, community metabolism caused small daily fluctuations in the pH levels (see supplementary material, Fig. S1). To assess the daily variability in the pH, measurements were performed in 30-min intervals using an automated pH logger (control system ACQ110 Aquatronica, Italy) over a 5-day period.

To achieve the ocean warming scenario, the water was heated in the enrichment tanks associated with the elevated-temperature mesocosms. Both pH (see supplementary material, Fig. S2a) and temperature (see supplementary material, Fig. S2b) were daily measured in each mesocosm during the 6-month experimental period using manual electrodes (Mettler Toledo SevenGo™ SG2, Australia; pH resolution: 0.01, pH accuracy: ± 0.01; temperature precision: 0.1 □C, temperature accuracy: ± 0.5 □C; calibrated on a daily basis). In addition, alkalinity and salinity were measured for each mesocosm in intervals of two weeks. Alkalinity was determined for a total of eight water samples per mesocosm tank using an automated titrator (888 Titrando, Metrohom, Switzerland), whereas salinity was measured using a hand-held refractometer for marine aquaria (SR6, Vital Sine). The partial pressure of CO_2 was calculated from the pH, temperature, alkalinity and

salinity measurements using the CO2SYS program for Excel (Pierrot et al., 2006) with constants from Mehrbach et al. (1973) as adjusted by Dickson & Millero (1987) (see supplementary material, Table S1).

4.2.3 Benthic habitats

Seagrass and rocky reef habitats were integrated within the simulated ecosystems in the form of eight circular habitat modules (four for each habitat) placed in each mesocosm tank. The seagrass modules were constructed from green polypropylene ribbon strips, attached to a plastic grid that was placed in a plastic tray (42 cm diameter), and mimicked the most abundant seagrass species in the region *Posidonia* spp. at a density of 305 strips m^{-2} (Bryars & Rowling, 2009) (see supplementary material, Fig. S3a). Following their construction, the seagrass modules were incubated *in situ* adjacent to natural seagrass beds for 14 days at ~2 m depth. The rocky reef modules (see supplementary material, Fig. S3b), were constructed from rocks and their attached biota collected *in situ* from 0.5 to 4 m depth using SCUBA diving and snorkeling. The rocks hosted a diverse assemblage of algae, dominated by *Sargassum* spp., *Cystophora* spp., a number of calcareous algae, and low growing fleshy algae such as *Exallosorus* spp. and *Zonaria* spp. The sessile animal constituents included sponges, tunicates, calcareous worms and some bivalves. Upon their collection, the artificial seagrass and rocky reef habitat were transported, fully submerged, to where the mesocosms were housed. Rocks hosting similarly-sized specimens and algal assemblages were positioned on plastic trays, identical to those used in the seagrass modules. Four artificial seagrass and four rocky reef islands were randomly assigned to each mesocosm where they were positioned on a mixed bed of inorganic and natural sand (see supplementary material, Fig. S4). The top 2-cm layer of the sandy substratum in the mesocosms consisted of sand that was collected *in situ* and harbored live meiofaunal communities.

4.2.4 Sample processing

For each habitat module of each habitat type in each mesocosm, one sediment sample for meiofauna density and species composition analyses was taken with a 2.5 cm diameter core down to a depth of 5 cm. Samples were washed

through a sieve with a mesh of 63 μm. After washing, the flotation method with colloidal silica solution (Ludox TM-50) adjusted to a specific gravity of 1.18 was used to separate organisms from the sediment (Heip et al., 1985). All meiofaunal organisms were counted on a Dolffus plate under a stereoscopic microscope in each sample; 120 nematodes were separated to be mounted in permanent slides. Nematodes were identified under an optical microscope at the lowest possible taxonomic level, by using the identification keys of Platt & Warwick (1983, 1988) and Warwick et al. (1998) and the NeMys database (Deprez et al., 2005).

4.2.5 Analyses of community descriptors

Species densities, species richness (number of species present in a sample), trophic diversity, and evenness of meiofaunal communities Pielou's index (J′) were calculated as representatives of community descriptors. The functional diversity of the nematodes was estimated using the index of trophic diversity (ITD) as 1 – ITD (Heip et al., 1985). The 1-ITD indicates the evenness of trophic guilds, as nematodes are divided into four functional trophic guilds, namely: bacterial or selective deposit feeders, non-selective deposit feeders, epistrate feeders that can scrape off microbes attached to sediment grains, and predators/omnivores (Wieser, 1953). The value of 1 – ITD ranged from 0.00 (lowest diversity, where one trophic guild accounts for 100% of the total nematode abundance) to 0.75 (highest trophic diversity, where four trophic guilds account for 25% of the total abundance each).

To evaluate significant differences in community descriptors between different habitats and climate scenarios, permutational multivariate analyses of variance PERMANOVAs was performed (Anderson, 2001). Multivariate data for species densities were transformed to Bray-Curtis distances to test for differences in multivariate metacommunity structure, whereas PERMANOVAs of univariate community descriptors were based on Euclidean distance matrices. Climate scenario was treated as a fixed factor composed by four distinct levels: Current conditions (C), Ocean acidification (OA), elevated temperature (T), as well the combined effect of acidification and warming (OA+T); habitat type (Ha) was also fixed with three levels (bare sand, artificial seagrass and rocky reef), while mesocosm (M) was considered as a random factor.

4.2.6 Elements of metacommunity structure (EMS)

The elements of metacommunity structure (EMS) were estimated and interpreted according to Leibold and Mikkelson (2002) and Presley et al. (2010). The EMS approach tests the fit of empirical data to at least twelve types of metacommunity structures according to three different metrics: coherence, turnover, and boundary clumping of species distribution (Fig.1). Through these idealized patterns, we can infer the underlying mechanisms structuring metacommunities. For example, metacommunities are randomly structured when species have distinct responses to multiple environmental gradients, whereas checkerboard pattern is usually generated via strong interspecific competition leading to mutual exclusion (Gascón et al., 2016). Nested patterns emerge when metacommunities are characterized by richness differences and the structuring mechanisms are inferred via their pattern of species loss (i.e., hyperdispersed, random, or clumped loss). When species have independent responses to the environmental gradient or dispersion abilities determine species range boundaries, metacommunity will have a random species loss, while hyperdispersed species loss may occur if tradeoffs between competitive ability and environmental tolerance exist (Gascón et al., 2016; Presley et al., 2010). If habitat specializations determine the species range boundaries across habitats within mesocosms, metacommunities might show a clumped species-loss pattern, with range boundaries coincident with ecotones (Presley et al., 2010). Evenly-spaced, Gleasonian, and Clementsian distributions are characterized by species turnover. Clementsian gradients (Clements, 1916) result in discrete compartments of species that replace each other as a group, while in Gleasonian gradients (Gleason, 1926) species show individualistic responses to the environmental gradient (Leibold & Mikkelson, 2002). For instance, if species competitive ability to exploit alternative resources determine their distribution, metacommunities would present an evenly spaced structure at the regional scale (Gascón et al., 2016; Henriques-Silva et al. 2013). Is important to keep in mind that metacommunities are influenced by multiple ecological processes, and the aim of EMS framework is to highlight the preponderant drivers of metacommunity structure, rather than point out which is the exclusive mechanism responsible for the observed pattern.

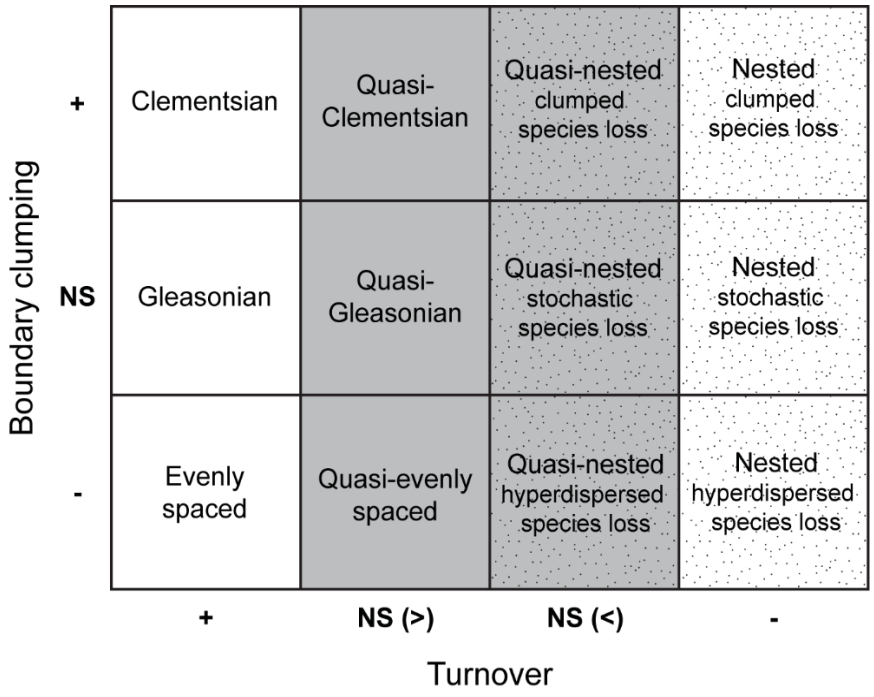

Fig. 1 Extracted from Presley et al. (2010). Twelve coherent metacommunity structures defined by range turnover and boundary clumping. Quasi-structures are shaded; nested structures that are distinguished by patterns of species loss are dotted. Significant positive results, +; significant negative results, –; non-significant clumping, NS, non-significant turnover but with more replacements than the average number in randomly generated metacommunities, NS (>); non-significant turnover but with fewer replacements than the average number in randomly generated meta- communities, NS (<).

The three EMS metrics were calculated from a presence – absence matrix, with replicate samples as rows and species presence/absence as columns. The interaction matrix was ordinated via reciprocal averaging (Gauch, 1982). The scores obtained from ordination can then be related to environmental or spatial variables (Presley & Willig, 2010). Coherence was measured by comparing the number of observed absences in the ordination matrix to the number of absences in randomized null matrices. A smaller number of embedded absences (Abs) than expected by chance indicates positive coherence, while many absences (i.e., absence is significantly larger than expected by the null models) indicates negative coherence. Significant positive coherence is indicative of nestedness, evenly spaced gradients, Gleasonian or Clementsian gradients (Leibold & Mikkelson, 2002). On the contrary, significant negative coherence indicates a checkerboard distribution, whereas a non-significant coherence suggests a random metacommunity and there is no need to proceed with other tests. Further analysis presupposes that community matrices have a positive coherence. Turnover was measured as the number of times one species replaced (Repl) another. Significant

negative turnover refers to nestedness, whereas significantly positive turnover (i.e., Repl is significantly larger than expected by chance) indicates evenly spaced, Gleasonian or Clementsian metacommunities. Significant positive coherence combined with a nonsignificant turnover can be interpreted as a quasi-structure (Presley et al. 2010). The evenly spaced, Gleasonian and Clementsian metacommunity types can be distinguished subsequently based on the index of boundary clumping (Leibold & Mikkelson, 2002). Boundary clumping was analyzed using Morisita's *I* dispersion index. Statistical significance was obtained by comparing the observed range boundary of samples against a chi-square distribution. When the Morisita's dispersion index is not different from 1, the range boundary is randomly distributed, which fits with a Gleasonian metacommunity structure. A Morisita's *I* significantly higher than 1 indicates a clumped range boundary (i.e., Clementsian metacommunity) and a value significantly lower than 1 indicates a hyperdispersed range boundary (i.e., evenly spaced metacommunity). Coherence (Abs) and turnover (Repl) indices were tested using the fixed-proportional null model "r1", where row sums are fixed (i.e., the species richness of each site was maintained), but column marginal frequencies (i.e., species frequencies of occurrence) were used as probabilities. Random matrices were produced based on 999 simulations and statistical significance of Abs and Repl was assessed by comparing the observed outcomes to the distribution of outcomes derived from the randomizations (Manly, 1995). Elements of metacommunity structure were evaluated based on axis 1 of reciprocal averaging. EMS analyses were done using the R package 'metacom' (Dallas, 2013) in the R environment (version 3.3.3, R Development Core Team 2017).

4.2.7 Habitat characteristics associated with structuring gradient

We used a least-square multiple regression with a backward stepwise selection to investigate the association between the EMS sample scores obtained from the reciprocal averaging ordination and a set of explanatory variables, including biomass of turf in rocky and seagrasses habitats, biomass of macrophytes on rocky habitat, chlorophyll-*a*, and detritus content on bare sand, and net primary production (see supplementary material Fig. S5). The most parsimonious model was chosen based on the lowest Akaike Information Criteria (AIC). Backward

regressions were performed using the R package ‘MASS’ (Venables & Ripley, 2002). The relative importance of explanatory variables for the linear model was calculated through the partitioning of R^2 by averaging over orders (Lindemann et al., 1980) using the R package ‘relaimpo’ (Grömping, 2006).

4.3 RESULTS

4.3.1 Local community descriptors

Fifty-four distinct nematode genera were identified (see supplementary material, Table S2). Dissimilarities among habitat communities varied as a response of climate stressors (pseudo-F = 3.162, p (perm) = 0.001, see also supplementary material Table S3). Temperature increased the similarity (t = 2.29, p (perm) = 0.001) in community structure among habitats as observed in the distance to centroids (Fig. 2a). Whilst the communities of all three habitats differed from each other under ambient conditions and ocean acidification, communities were similar (irrespective of microhabitat) in the T and OA+T treatments (see supplementary material Table S4).

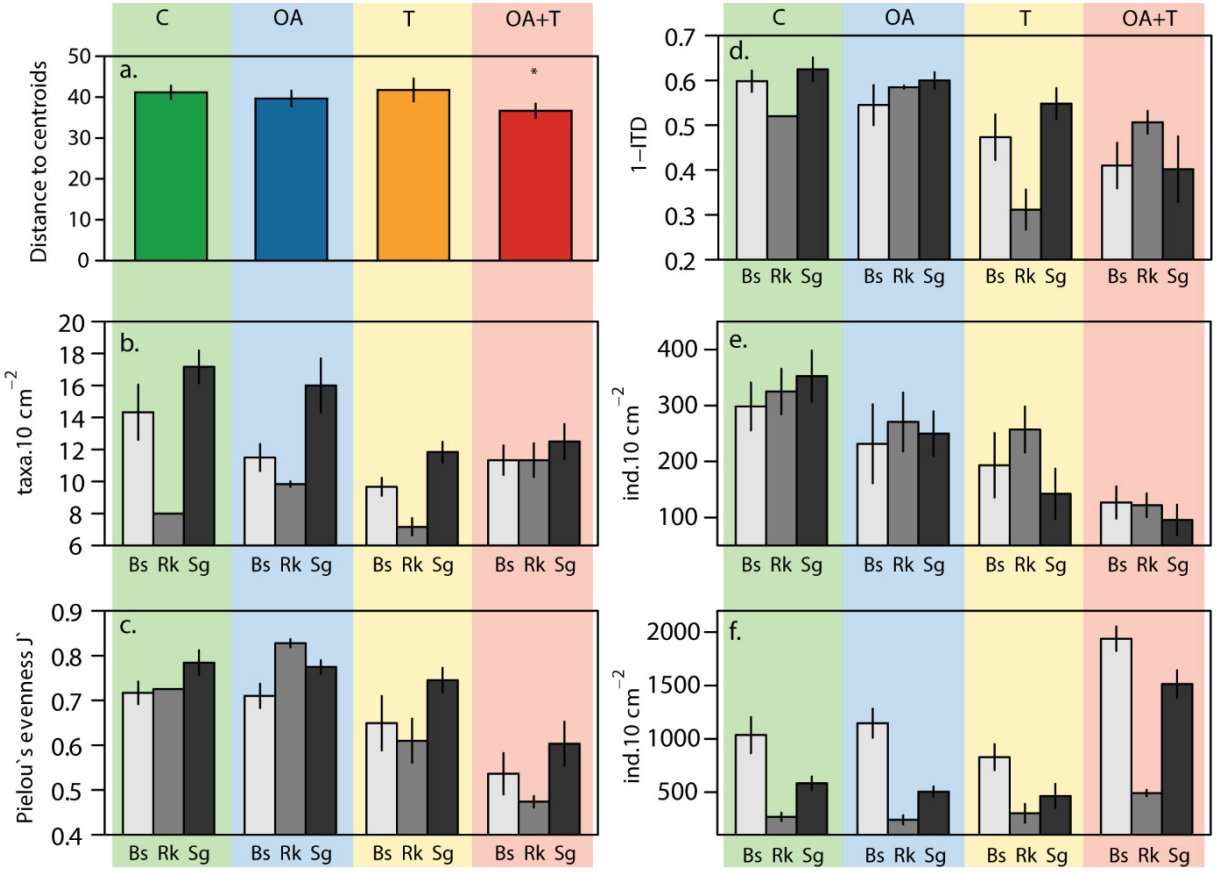

Fig. 2 Average (mean ± SE) of univariate community descriptors in each climate scenario. Number of nematode genera (a.); Pielou's evenness (b.); index of trophic diversity 1-ITD (c.); total density of copepods (d.); total density of nematodes (e.). Bars represent habitats: bare sand habitats in light gray (Bs); rocky reef in gray (Rk); and seagrass in dark gray (Sg).

There was a decrease in biodiversity of metacommunity under warming and the combined effects of temperature and acidification. Nematode richness was significantly lower in T (t = 4.43, p(mc) = 0.001) and OA+T (t = 2.64, p(mc) = 0.021) compared to control (C). In addition, habitats exposed to a combined increase in temperature and ocean acidification (OA+T) showed a similar number of taxa. Under present conditions (C) and ocean acidification (OA), the number of taxa was always higher in seagrass, followed by bare sand and rocky reefs in the other treatments (Fig. 2b; supplementary material, Table S3 and S4).

Pielou's evenness was significant lower in OA (t = 2.32 and p(mc) = 0.042), T (t = 2.15 and p(mc) = 0.051) and OA+T (t = 7.90 and p(mc) = 0.001) compared to control. In this case, habitats showed a similar response across all treatments, with lower evenness in bare sand and rocky reefs compared to artificial seagrass habitat (Fig. 2c; supplementary material, Table S4).

There was a significant decrease in meiobenthic metacommunity function, corroborated by the lower values of the index of trophic diversity 1-IDT in all three habitats within T ($t = 3.85$ and $p(mc) = 0.005$) and OA+T ($t = 3.42$ and $p(mc) = 0.016$) compared with OA and control scenarios (Fig. 2d). The relative abundances of diatom feeders and predators were lower in T and OA+T compared to ambient conditions (supplementary material, Table S4). In addition, copepod densities, an important group of secondary producers, were significantly lower in all three habitats within T ($t = 3.08$ and $p(mc) = 0.012$) and OA+T ($t = 6.21$ and $p(mc) = 0.001$) compared to control (Fig. 2e; supplementary material, Table S3).

4.3.2 Elements of metacommunity structure EMS

The overall nematode metacommunity fits into a nested structure with clumped species loss (Table 1), which means the metacommunity was structured mainly by species richness differences among climate treatments and habitats (Table 1). Significantly positive coherence, negative turnover, and clumping values >1 indicating that the nematode species showed group responses to climate change treatments. More species occur under current climate conditions with species richness declining with increase in warming and acidification, resulting in a nested structure (Fig. S6, Table 1).

Metacommunities from C, OA, and T had a clumped pattern of species loss, with range boundaries associated with the coastal sedimentary habitats (i.e., rocky and bare sand communities are nested subsets of species pool from seagrass habitat). However, metacommunity in OA+T was randomly structured. The coherence of metacommunity exposed to the combined effects of acidification and warming was not significant (i.e., the number of absences present in the ordinated matrix did not differ from those expected by chance). In this case species distributions are regulated by multiple independent environmental drivers (Table 1).

Table 1 Summary results of the elements of metacommunity analysis (EMS) for the overall model including all treatments as well for each metacommunity exposed to a distinct climate scenario. Simulated mean (Sim), embedded absences (Abs), and replacement (Repl). Metacommunity structure: nested pattern with clumped species loss (NCSL); and random pattern (Random).

	Coherence			Turnover			Clumping		Structure
	Sim	Abs	z	Sim	Repl	z	*df*	Morisita's	
Overall	1953	1312	5.54***	108500	51906	11.57***	51	6.79***	NCSL
C	322	214	3.85***	6998	3215	6.14***	42	6.82***	NCSL
OA	282	203	3.20***	6310	2334	7.12***	38	5.55***	NCSL
T	224	166	2.79**	4814	2724	5.35***	30	3.14***	NCSL
OA+T	247	212	1.82	-	-	-	-	-	Random

Significance codes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

4.3.3 Habitat characteristics structuring meiobenthic metacommunity

The most parsimonious model according to Akaike Information Criteria (AIC) (see supplementary material, Table S4) indicated that the underlying structure of nematode metacommunity was positively correlated with net primary production (R^2 = 21.20%) followed by chlorophyll-*a* (R^2 = 20.51%) and detritus content (R^2 = 19.93 %), and by a negative correlation (R^2 = 17.25%) with macrophyte biomass (Table 2).

Table 2 Summary results of the least squares multiple regression testing the relationships between elements of metacommunity (EMS) site scores and changes in mesocosms characteristics. Relative importance of explanatory variables is shown in percentages. Coefficients are shown with their standard deviation (n = 12).

	df1	*df2*	*F*	R^2
	6	5	38.21***	97.87
	Coefficients		t-value	% of R^2
Turf biomass on Rock	0.006 ± 0.003		2.011	10.34
Detritus	0.033 ± 0.007		4.570	19.93
Macrophyte biomass on Rock	-0.009 ± 0.002		-3.599	17.25
Turf biomass on Seagrass	-0.007 ± 0.002		-3.274	10.73
Chlorophyll-*a* on Bare sand	0.136 ± 0.030		4.555	20.51
Net primary production	0.307 ± 0.157		1.957	21.20
(Intercept)	-1.419 ± 0.351		-4.040	

Significance codes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

4.4 DISCUSSION

Our experiment demonstrates a homogenization of species communities across habitats under projected climate change. Community homogenization is evident at broader spatial scales under elevated temperature (García Molinos et al., 2015). For terrestrial and marine vertebrate fauna this phenomenon often results

from latitudinal range expansions of generalist species (Davey et al., 2012; Magurran et al., 2015), but the effects on dynamics and species distribution across adjacent habitats at smaller spatial scales largely remain unexplored (Urban et al., 2012). Elevated CO_2 will increase productivity homogenizing food availability among habitats, while warming will exclude sensitive species leading to a facilitation of resistant non-selective deposit feeders. Such biodiversity homogenization could affect ecosystem functioning with consequent cascading effects on marine food-webs.

Under strong environmental gradients, metacommunities are non-randomly structured (Gascón et al., 2016), but the increase in primary and detritus production promoted by climate change appears to alter meta-communities to randomly structured. We observed a common trend of nestedness in meiobenthic metacommunity structure. Local communities are structured by habitat constraints where species pool present in artificial seagrasses were distributed in poorer subsets along bare sands and rocky reefs. However, in the combined treatment of warming and acidification metacommunity lost coherence, showing a high degree of randomness. When metacommunities are randomly distributed, there are no clear gradients or discernible patterns in species distributions (Heino et al., 2015; Leibold & Mikkelson, 2002). Moreover, the decrease in coherence suggests that nematodes responded differently to multiple environmental gradients (Dümmer et al., 2016; Presley et al., 2010). The synergistic effect of warming and acidification affect the entire system, undermining spatial variations in the food availability among habitats.

Homogenization and randomness of communities were corroborated by the overall decrease in richness (i.e., among and within habitats). For instance, local avian diversity increased under climate change at high latitudes (Davey et al., 2012). Fish richness on the contrary, did not decrease despite the changes in community structure and composition (Magurran et al., 2015). The loss of local biodiversity has been associated to the fact that climate change can affect diversity by changing species interaction causing extinctions at local scales (García Molinos et al., 2015; Zeppilli et al., 2015). In the present study, the decrease on richness in seagrasses and rocky reefs allowed the dominant taxa of bare sands, *Anticoma* and *Synonema,* to dominate all habitats. These two genera tolerated and prospered under climate change, benefiting from the decrease in competition due to local extinctions.

The decrease in regional species richness (ɣ-diversity) promoted also a decrease in nematode functional diversity. In addition, copepod density decreased in scenarios of warming and ocean acidification. These changes may have substantial effects on the coastal food web (Gingold et al., 2013; Zeppilli et al., 2015). Changes on biomass transfer from benthos to demersal fishes and increases in the amount of biomass flowing through microbial loop can generate regime shifts with consequent homogenization and biodiversity loss on short scales of time and space (Goldenberg et al., 2017; Magurran et al., 2015).

In marine ecosystems, climate change is associated with shifts in several variables and processes such as temperature, circulation, stratification, nutrient input, oxygen content, and ocean acidification, with potentially wide-ranging biological effects (Doney et al., 2012). The synergistic effects of multiple stressors may modify energy flows as well as biogeochemical cycles, eventually impacting the overall ecosystem functioning and services upon which people and societies depend (Doney et al., 2012; Nagelkerken & Connell, 2015). Climate change will favour even more the dominant generalist species that feed directly and indirectly upon bacteria and/or associated unicellular microeukaryotes while predators and copepod densities will decrease. The observed changes in dominance, combined with the homogenization and decreasing in biodiversity drive changes in metacommunity structure. Species pool of coastal areas will be no longer regulated by habitat's filtering, and will be restructured by the dispersion of the tolerant species (i.e. random metacommunities).

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SUPPLEMENTARY MATERIALS

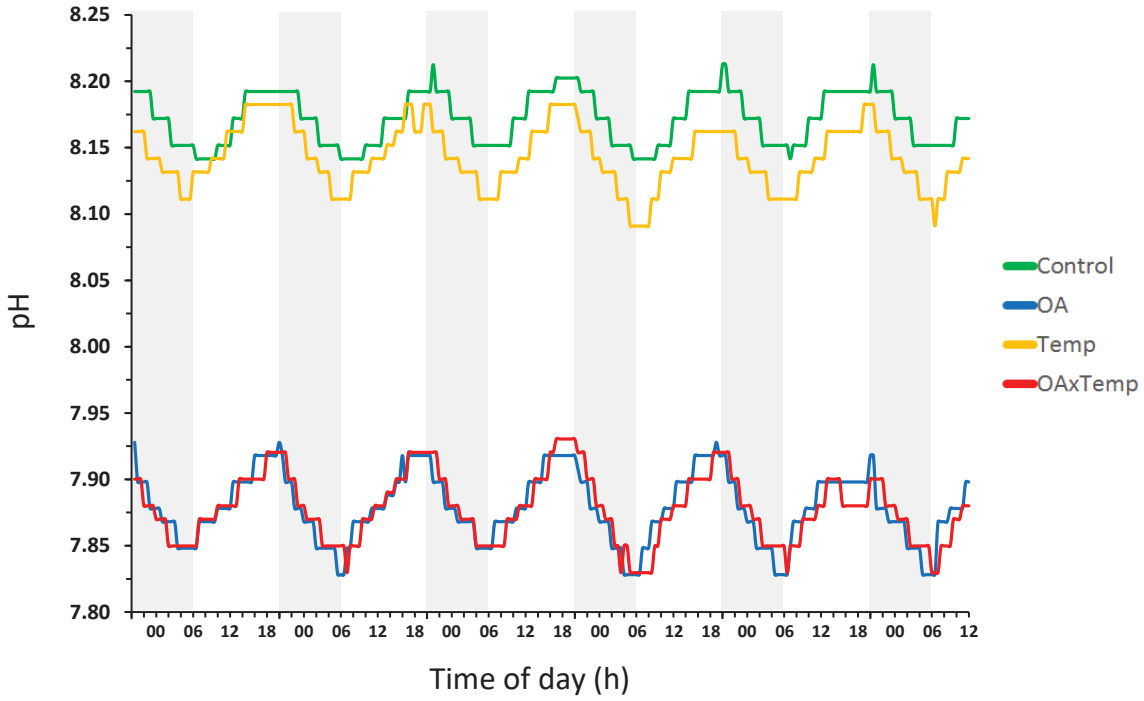

Figure S1. Daily variation in pH due to the community's metabolism as would be expected in natural systems. Night times are identified by the grey bands and mark and white bands day-time.

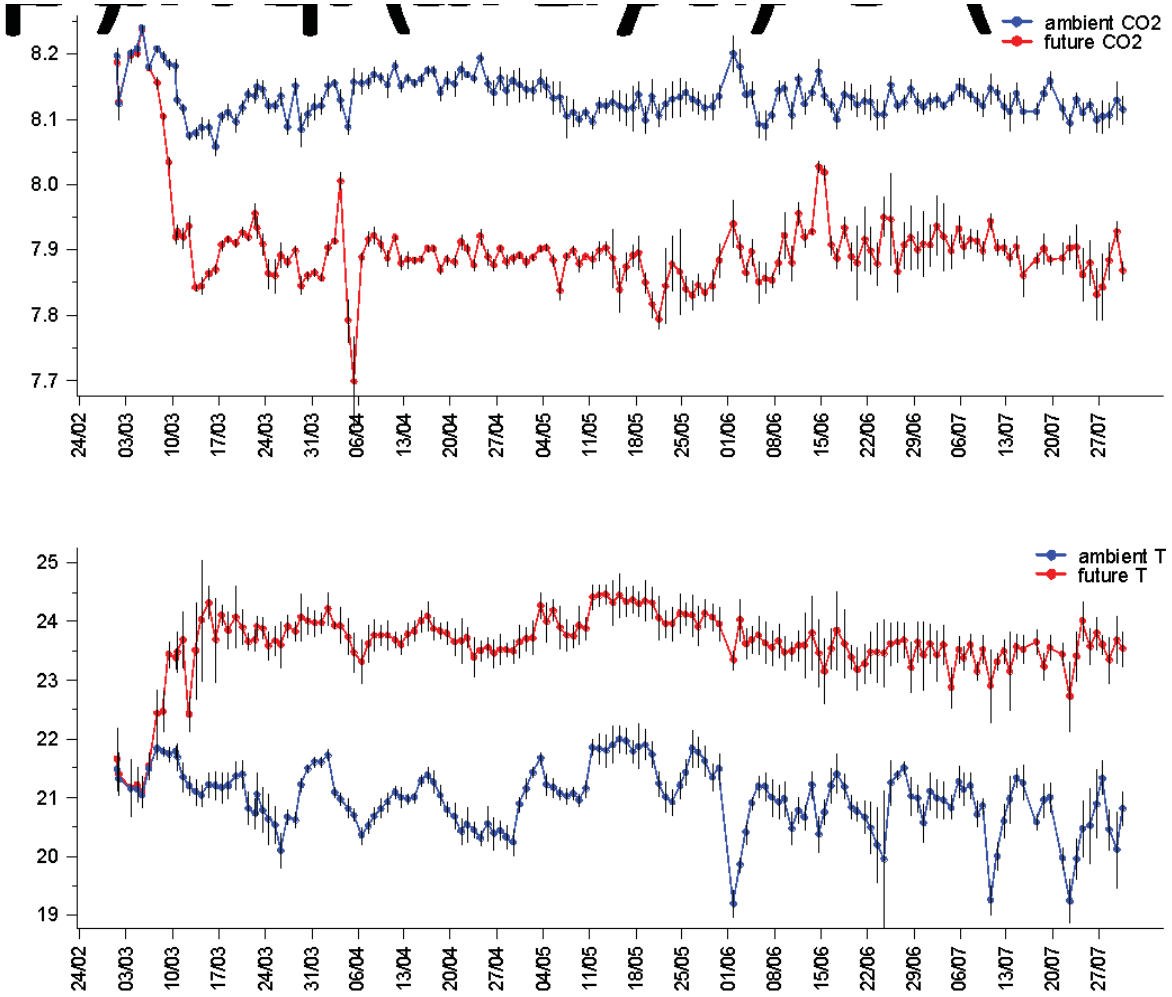

Figure S2. The variation in (a) pH (mean ± SD) and (b) temperature (mean ± SD) in all treatments over a period of five months. Red lines show the future scenarios treatments and blue ambient treatments.

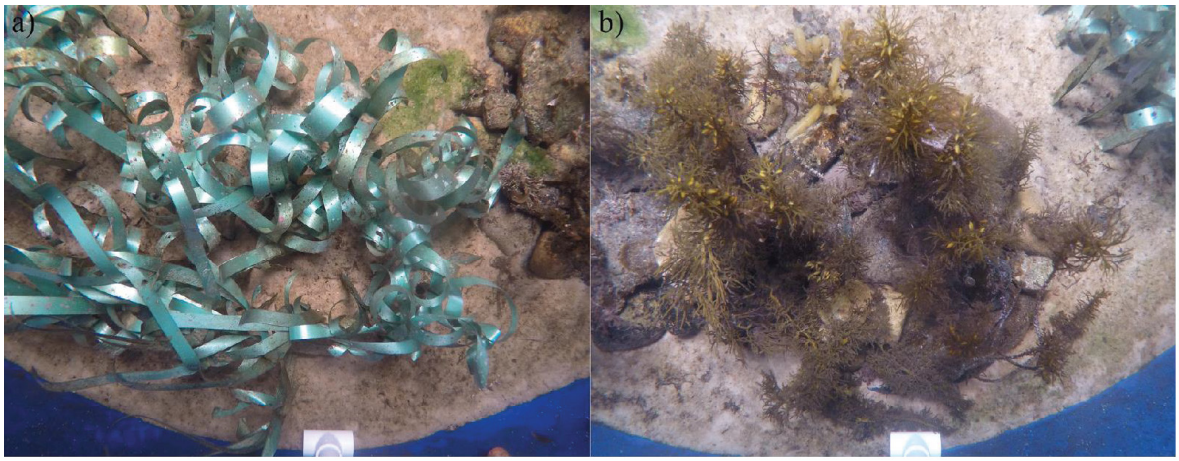

Figure S3. The two main structural components of the mesocosm (a) artificial seagrass and (b) live rocky reef.

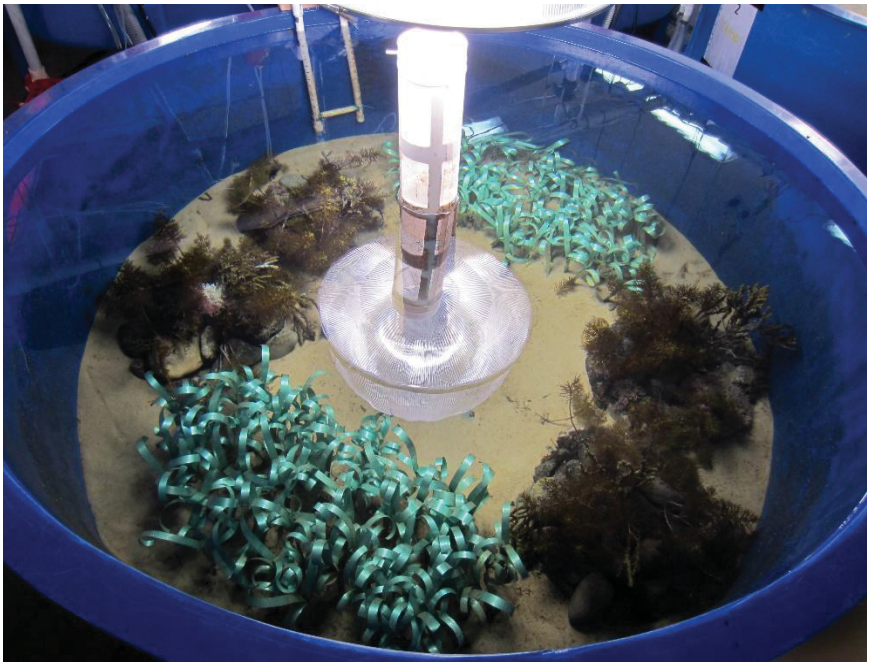

Figure S4. Mesocosm, composed by three distinct habitats (bare sand, seagrass and rocky reef).

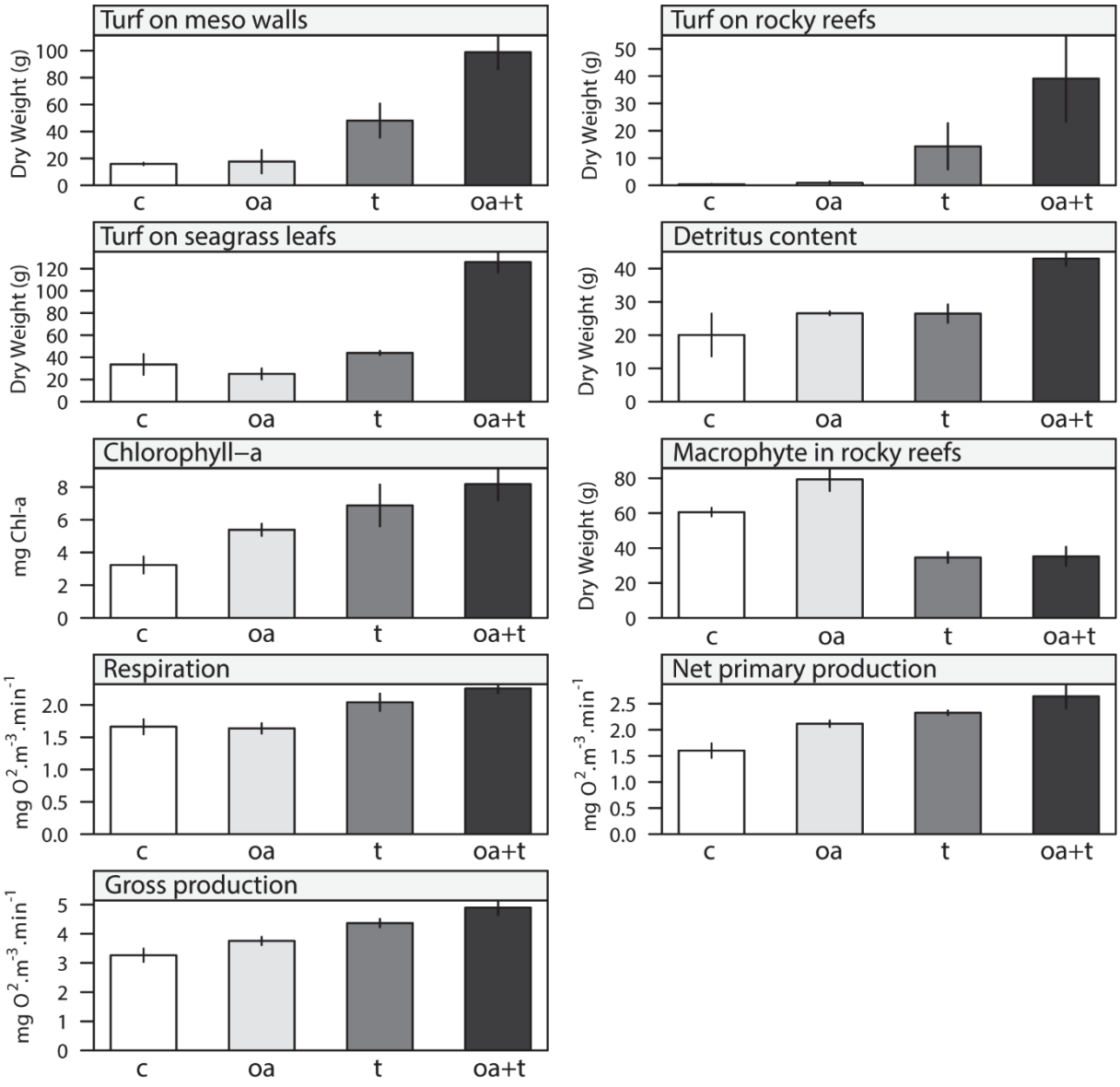

Figure S5. Effects of ocean acidification and warming on ecosystem's characteristics (mean ± SE).

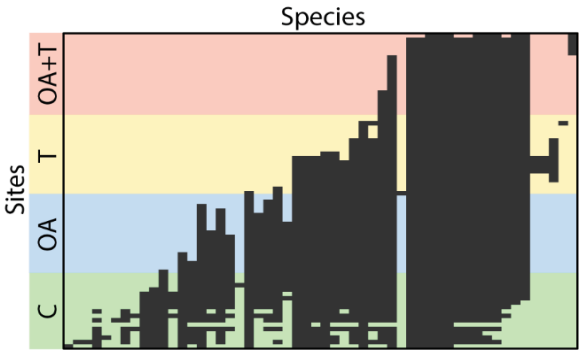

Figure S6. Visualization of the structuring gradient of species distributions in response to climate change, with samples as rows (within treatments) and species as columns. Black rectangles indicate species occurrence. C= Current conditions, OA = ocean acidification, T = warming, OA+T = warming and acidification combined.

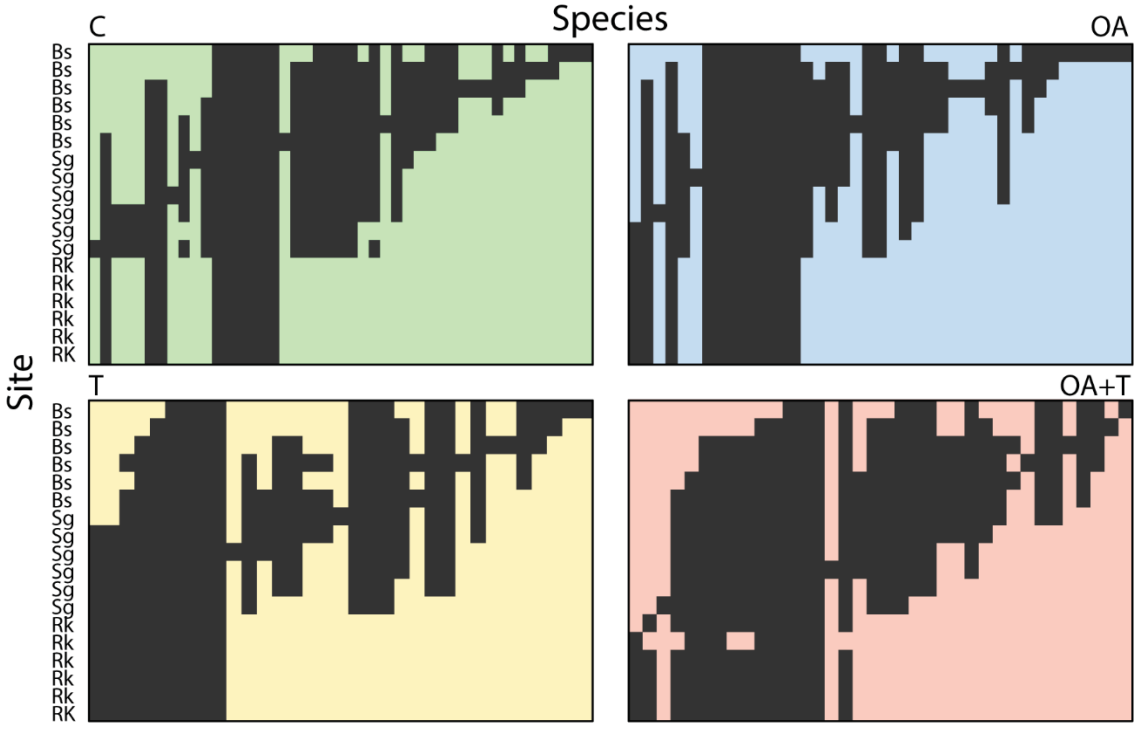

Figure S7. Visualization of the structuring gradient of species distributions in response to habitats within each climate scenario. Sites are rows and species are columns. Black rectangles indicate species occurrence. Current conditions (green), ocean acidification (blue), warming (orange), warming and acidification together (red). Habitats: bare sand (BS); seagrass (Sg); and rocky reefs (Rk).

Table S1. Chemical water parameters (mean ± SD) over the treatment period.

Parameter	**C**	**OA**	**T**	**OAT**
Temperature (°C)	21.0 ± 0.14	20.9 ± 0.04	23.7 ± 0.19	23.7 ± 0.08
pH	8.14 ± 0.004	7.89 ± 0.009	8.12 ± 0.002	7.89 ±0.009
Salinity (ppt)	36.3 ± 0	36.3 ± 0	36.3 ± 0	36.3 ± 0
Total Alkalinity (μmol kg^{-1})	2482 ± 4	2485 ± 5	2486 ± 6	2493 ± 3
pCO_2 (ppm)	465 ± 5	905 ± 6	500 ± 8	915 ± 25
HCO_3 (μmol kg^{-1})	1995 ± 6	2186 ± 3	1985 ± 2	2166 ± 9
CO_3 (μmol kg^{-1})	200 ± 2	123 ± 1	206 ± 2	135 ± 3
Ω Calcite	4.74 ± 0.05	2.91 ± 0.02	4.90 ± 0.05	3.20 ± 0.07
Ω Aragonite	3.09 ± 0.04	1.90 ± 0.01	3.22 ± 0.03	2.10 ± 0.05

Table S2. Average density and relative abundance of nematode genera in climate treatments (n=18, mean ± SD).

	Density (ind.10cm^{-2})				Relative Abundance (%)			
Genus	**C**	**OA**	**T**	**OA+T**	**C**	**OA**	**T**	**OA+T**
Acanthopharynx	0±0	0±0	4±10	4±11	0±0	0±0	1±2	0±1
Anticoma	222±196	228±196	252±251	715±388	30±16	33±10	41±16	55±13
Bathylaimus	0±0	1±4	0±0	0±0	0±0	0±0	0±0	0±0
Bendiella	0±0	0±0	0±2	0±0	0±0	0±0	0±0	0±0
Catanema	0±0	0±0	0±0	3±11	0±0	0±0	0±0	0±1
Chromadora	29±36	31±35	10±17	11±19	4±5	5±5	2±2	1±1
Chromadorella	16±14	8±6	1±2	6±12	4±5	2±2	1±1	1±1
Chromadoridae	1±2	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Chromaspirina	0±2	0±0	0±2	4±12	0±0	0±0	0±0	0±1
Comesomatidae	2±5	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Cyatholaimus	73±61	40±32	22±18	67±60	20±22	11±12	6±7	10±14
Daptonema	3±9	3±6	3±12	4±9	1±2	0±1	1±2	0±0
Desmodora	1±2	1±3	0±0	1±6	0±0	0±0	0±0	0±0
Dichromadora	5±12	1±4	3±11	0±0	1±1	0±1	0±1	0±0
Diplopeltis	0±1	1±3	0±0	0±0	0±0	0±0	0±0	0±0
Dorylaimopsis	2±5	1±3	4±7	6±13	0±1	0±0	1±2	0±1
Dracogalerus	1±3	2±3	1±3	0±0	0±0	1±1	0±1	0±0
Enoplus	0±0	0±0	0±0	2±10	0±0	0±0	0±0	0±0
Eurystomina	10±12	9±14	3±6	23±33	2±2	1±2	1±1	1±2
Graphonema	6±7	14±14	3±3	6±11	1±2	4±5	1±2	1±1
Halalaimus	0±2	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Halichoanolaimus	0±0	1±3	0±0	0±0	0±0	0±1	0±0	0±0
Laimella	0±2	1±3	0±0	1±5	0±0	0±0	0±0	0±0
Marylynnia	12±18	18±26	21±48	39±81	1±2	3±5	5±9	2±5
Mesacanthion	1±5	0±2	0±0	2±5	0±1	0±0	0±0	0±0
Metalinhomoeus	6±14	1±2	2±7	6±16	1±2	0±0	1±3	0±1
Metoncholaimus	4±6	6±13	3±7	2±7	0±1	1±2	1±1	0±0
Meyersia	5±14	0±0	0±0	0±0	0±1	0±0	0±0	0±0
Microlaimus	1±3	3±7	5±9	17±41	0±0	1±1	1±2	1±2
Nannolaimus	0±0	1±6	0±0	1±6	0±0	0±1	0±0	0±0
Nemanema	2±5	1±3	0±0	1±3	0±0	0±0	0±0	0±0
Nicascolaimus	1±4	1±3	0±1	0±0	0±0	0±0	0±0	0±0
Onchium	1±5	1±3	0±0	0±0	0±1	0±0	0±0	0±0
Oncholaimus	30±28	22±22	19±36	19±33	4±3	4±2	2±4	1±2
Onyx	1±2	1±4	1±4	1±3	0±0	0±1	0±1	0±0
Paracanthonchus	1±3	2±6	0±0	4±7	0±0	0±1	0±0	0±1
Perspirinia	0±2	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Pomponema	1±3	1±3	2±5	5±13	0±0	0±0	1±3	0±1
Richtersia	0±2	0±0	1±2	0±0	0±0	0±0	0±1	0±0
Robbea	0±0	0±0	0±0	1±2	0±0	0±0	0±0	0±0
Sabatieria	0±2	1±3	0±0	0±0	0±0	0±0	0±0	0±0
Spilophorella	53±68	81±168	25±30	70±123	6±7	8±11	4±7	5±7
Spirinia	3±8	1±4	0±1	2±5	1±2	0±1	0±0	0±0
Steineria	2±5	4±7	0±0	5±12	0±0	1±2	0±0	0±1
Symplocostoma	8±7	3±8	5±15	7±14	1±1	0±1	1±2	0±1
Synonema	74±87	100±106	114±105	213±322	11±5	16±10	25±20	12±17
Syringolaimus	4±15	10±38	5±14	14±35	0±2	1±3	1±2	1±2
Terschellingia	5±12	0±1	0±1	9±34	1±2	0±0	0±1	1±2
Theristus	16±29	11±22	9±15	8±15	2±2	1±2	1±2	0±1
Viscosia	24±23	17±20	10±19	32±37	4±2	3±2	1±2	2±2
Xennella	1±2	0±0	1±3	0±0	0±0	0±0	0±0	0±0
Xyallidae	0±0	0±1	0±0	0±0	0±0	0±0	0±0	0±0
Zalonema	1±3	1±3	1±2	0±0	0±0	0±1	0±1	0±0

Table S3. Results of the multivariate and univariate permutational analyses of variance PERMANOVA for nematode community, number of nematode taxa. Pielou's evenness J of nematode community, total nematode and copepod densities and index of trophic diversity 1-ITD. df = degrees of freedom, MS = mean squares, (n=2).

		Metacommunity		Richness		Pielou's Evenness	
	df	MS	Pseudo-*F*	MS	Pseudo-*F*	MS	Pseudo-*F*
Sc	3	7223.4	6.074***	43.815	7.371**	0.195	23.076***
Ha	2	21734.0	18.276***	168.010	28.264***	4.029^{E-2}	4.772*
Sc x Ha	6	3761.4	3.162***	20.218	3.401*	1.433^{E-2}	1.697
Me (Sc x Ha)	24	1189.2	1.700***	5.944	1.014	8.443^{E-3}	1.399
Residual	36	699.4	-	5.861	-	6.033^{E-3}	-
Total	71	-	-	-	-	-	-
		1-ITD		Copepod density		Nematode density	
	df	MS	Pseudo-*F*	MS	Pseudo-*F*	MS	Pseudo-*F*
Sc	3	0.113	8.594**	141670.0	10.914***	2.354^{E+6}	25.904***
Ha	2	0.024	1.831	8389.0	0.646	4.986^{E+6}	54.874***
Sc x Ha	6	0.036	2.719*	6605.9	0.509	3.204^{E+5}	3.526*
Me (Sc x Ha)	24	0.013	2.082*	12981.0	1.154	90869	2.169*
Residual	36	0.006	-	11248.0	-	41893	-
Total	71	-	-	-	-	-	-

Significance codes: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Where there was significant overall *F*-ratio comparing treatments ($P < 0.05$, 9999 permutations), pairwise comparisons were done. Numbers correspond to habitats, bare sand (1); seagrass (2); and rocky reef (3); underlining bars indicate habitats that were not statistically significantly different ($P < 0.05$) according post-hoc pairwise comparisons.

Table S4. Pair-wise comparisons for the term Sc x Ha testing for differences among habitats across the four climate scenarios.

	Metacommunity structure							
	CC		OA		T		OA+T	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
sg-bs	2.21	0.010	2.57	0.002	1.47	**0.099**	1.33	**0.317**
sg-rk	4.15	0.002	3.18	0.005	1.35	**0.172**	3.05	**0.106**
bs-rk	4.66	0.001	3.79	0.003	1.98	0.024	4.30	**0.095**
	Nematode richness							
	CC		OA		T		OA+T	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
sg-bs	1.38	**0.218**	3.58	0.022	1.74	**0.150**	0.61	**0.577**
sg-rk	6.44	0.005	7.89	0.002	4.54	0.004	0.64	**0.547**
bs-rk	4.27	0.015	1.64	**0.172**	2.65	0.048	0.69	**0.123**
	1-ITD							
	CC		OA		T		OA+T	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
sg-bs	0.88	**0.406**	2.72	**0.057**	0.84	**0.477**	0.07	**0.943**
sg-rk	4.44	0.021	0.73	**0.513**	3.50	0.028	0.89	**0.429**
bs-rk	4.17	0.019	6.93	0.002	1.78	**0.146**	2.51	**0.071**
	Nematode total density							
	CC		OA		T		OA+T	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
sg-bs	2.26	0.049	3.04	0.038	1.83	**0.132**	2.35	**0.088**
sg-rk	3.26	0.039	3.29	0.037	0.83	**0.454**	15.68	0.001
bs-rk	4.03	0.015	4.30	0.012	2.67	**0.052**	8.42	0.004

Table S5. Analysis of deviance for the backward stepwise multiple regression model of site scores obtained from the ordinated matrix and ecosystem's characteristics.

Step	***df***	**Dev Resid**	***df***	**Resid Dev**	**AIC**
			3	0.10132	-39.29146
- Gross Production	0	0.000	3	0.10132	-39.29146
- Turf biomass in wall	1	8.696^{E-7}	4	0.10133	-41.29135
- Respiration	1	4.021^{E-8}	5	0.10535	-42.82447

Final model: site scores ~ turf biomass (rock) + detritus + macrophyte biomass (rock) + turf biomass (seagrass) + chlorophyll-*a* (sand) + net primary production

5 GENERAL DISCUSSION

Soft-bottom marine landscapes are primarily shaped by physical and geomorphological processes (Zajac 2008). Horizontal/advection and vertical/diffusion water movements introduce variability in the seafloor topography and sediment composition over multiple spatial scales (Zajac 2008, Chapman et al. 2010). In addition, bioengineering and bulldozing modify sediment matrix, increasing heterogeneity and patchiness (Meysman et al. 2006, Kristensen et al. 2012). Transitional gradients between terrestrial and marine ecosystems influence the coastal environment (Thrush et al. 2013, Valanko et al. 2015). However, we still trying to understand how metacommunities are organized and which are the underlying mechanisms structuring them at the coastal landscape.

Throughout this thesis I explored three main issues of metacommunity ecology: 1) the structure of metacommunities in dynamic heterogeneous landscapes; 2) the effect of long-term changes in metacommunity dynamics; and 3) the importance of small-scale spatial and temporal variability for monitoring metacommunities.

Using the three topics above as guidelines for generalizations, I bring below an overview of most relevant findings, as well the relationships among all the works presented in the thesis. Moreover, I examine the implications of these findings for future studies, pointing out further directions.

5.1 METACOMMUNITY ORGANIZATION AND DYNAMICS IN HETEROGENEOUS LANDSCAPES

Heterogeneous landscapes of coastal regions, are formed by a configuration of different habitats, where the interplay between environmental gradients and spatial constraints are determinant for species distributions (Cottenie 2005, Lohrer et al. 2013, Datry et al. 2016). This is particularly evident for ecological communities at dynamic ecosystems, such estuaries, wetlands, sandy beaches, temporary pounds, streams, lakes, etc. (Vanschoenwinkel et al. 2007, Heino et al. 2015, Valanko et al. 2015, Dümmer et al. 2016, Gascón et al. 2016). Consequently, adjacent habitats host contrasting communities, influenced by different processes

and controlled by distinct mechanisms, which generate different patterns of variation (chapters 2, 3).

Mangroves are less exposed and hydrodynamic energy is low, increasing spatial constraints and small-scale spatial variability in fauna distribution (chapter 2). In this habitat, species composition is determined by biogeochemical gradients (e.g., aerobic and anaerobic respiration profiles) and by the tolerance to hypoxia due to the high amount of organic matter deposition (Mermillod-Blondin and Rosenberg 2006, Vieira and Fonseca 2013). Consequently, environmental filtering is high, and communities inhabiting mangrove sediments have low turnover being composed always by a similar group of species. Variations in local communities are mainly related to differences in species richness, suggesting that patch quality drives diversity which in turn may generate nestedness in metacommunity distribution (Fig.1a). At larger spatial scales though, we may observe a decoupling between nematode richness variations and mangrove landscape characteristics (chapter 1). Therefore, nematode diversity from mangrove sediments might be structured by colonization/extinction and patch-dynamics at small and intermediate spatial scales (10 cm to 100 km), while at large scales (> 1000 km) neutral processes and spatial constraints increase in importance (Leibold and Loeuille 2015, Dümmer et al. 2016).

Estuarine tidal flats are exposed to intermediate levels of hydrodynamic energy when compared to mangrove and sandy beaches. At this habitat, nematodes are mainly structured by gradients in salinity, temperature, sediment texture, and by the amount and quality of food available, e.g., detritus, fresh microphytobenthic, and microbial mats (Franco et al. 2007, Braeckman et al. 2015, Valanko et al. 2015). Differences in species osmotic tolerances, grain size and food preferences act together over multiple spatio-temporal scales shaping community structure and composition (chapters 2, and 3). Moreover, species turnover among local communities is high and metacommunity shows a Clementsian pattern, structured by species-sorting mechanisms (Brown et al. 2017). These findings are consistent with the notion that niche-based patterns such as Clementsian (Fig.1b) and Quasi-Clementsian may emerge from ecological communities living under strong environmental gradients (Gascón et al. 2016).

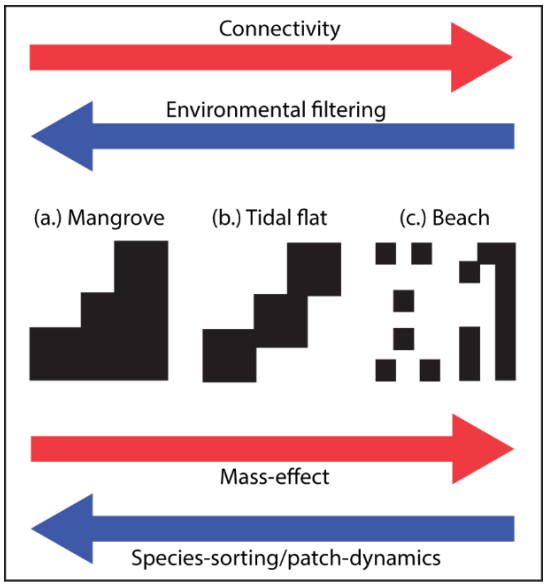

Fig 1. Conceptual dynamic of nematode metacommunities at coastal ecosystem linking patterns, processes and mechanisms. Mangrove vegetated tidal flats hosts nematode communities with nested/clumped species loss pattern of distribution (a.), communities at estuarine tidal flats have a clementsian distribution (b.), and nematode are randomly distributed at sandy beaches (c.).

Conversely, in sandy beaches physical/advective forces are important for community structure and dynamics (Gallucci and Netto 2004, Defeo and McLachlan 2005, Gheskiere et al. 2005). Connectivity is high, and dispersal prevents species extinctions at local communities (chapter 3). Consequently, sandy beaches are also more variable through time. These differences lead to a higher temporal variation in community structure compared with temporal variation in the more protected coastal habitats (chapter 2). Furthermore, small-scale spatial variability is lower and metacommunity is structured by mass-effect mechanisms (chapter 3). These mass-effect dynamics associated to hydrodynamics intensity leads to randomness in metacommunity organization (Fig.1c). In this case, species shows independent responses to multiple drivers (Presley et al. 2010) and passive dispersal overwhelm environmental filtering (Defeo and McLachlan 2005, Vanschoenwinkel et al. 2007). These findings might have important implications for the resilience of marine communities to habitat fragmentation, pollution and climate change disturbances (Thrush et al. 2008, Urban et al. 2012, Chave 2013). Therefore, gather empirical evidence about biological interactions (e.g., colonization/succession dynamics and competition) across multiple scales will bring further advances in metacommunity theory (Chave 2013, Richardson et al. 2014). Incorporating the responses of species and communities' interactions in our models of biodiversity distribution and

dynamics will improve the predictive power about the potential effects of human impacts on dynamic ecosystems.

5.2 PREDICTING LONG-TERM CHANGES IN BIODIVERSITY AND METACOMMUNITY DYNAMICS

Adjacent ecological communities are constantly sharing species (Leibold et al. 2004b, Urban et al. 2008). Although we barely know how metacommunities will respond to long-term changes decurrent from climate (Urban et al. 2012), at large spatial scales (e.g., latitudinal gradients) there are recent predictions accounting for a homogenization in species diversity and composition due to warming (Davey et al. 2012, Magurran et al. 2015). Despite this, our predictive understanding of these changes still limited by the scarce availability of experimental outcomes evaluating the effects of climate-change over multiple habitats and communities simultaneously (chapter 4).

In dynamic environments such as the soft-bottom aquatic regions of coastal ecosystems, warming and acidification of water drive changes in metacommunity structure and dynamics, causing regional extinctions (γ-diversity) and decreases in species beta-diversity (chapter 4). Warming and acidification changes the environmental filtering and sensitive species are excluded either by physiological constraints or via interspecific competition. In addition, climate change promotes homogenization in primary and microbial production among coastal habitats (Goldenberg et al. 2017), favoring tolerant species with generalist/opportunistic food preferences (chapter 4). Based on these results we expect that metacommunities structured by niche-based dynamics at current conditions (chapter 2) will present a random pattern of distribution in future scenarios of warming and ocean acidification (chapter 4, Fig. 2). Furthermore, environmental filtering will be overruled by dispersion and homogenization among habitats at the landscape scale, over short and intermediate periods of time (García Molinos et al. 2015).

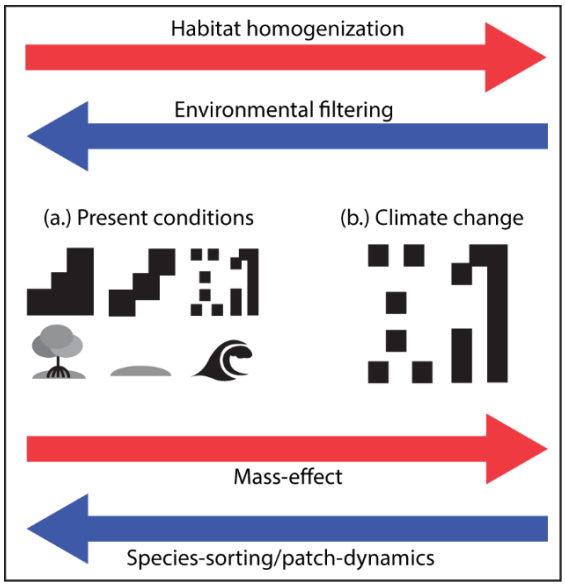

Fig 2. Conceptual changes in dynamic of nematode metacommunities at coastal ecosystem under different climate scenarios. Nematode communities are structured by a diversity of mechanisms in a more niche-based dynamic under natural conditions (a.). Conversely, communities are randomly distributed under climate change scenarios of habitat homogenization (b.).

The interactive effects of colonization/succession dynamics and competition will act in the opposite direction preventing adaptation of nonresident species to new niches (Urban et al. 2008, 2012). These effects will be more intense on highly diverse communities (Urban et al. 2008, Richardson et al. 2014) dominated by strong environmental gradients (Valanko et al. 2015, Gascón et al. 2016), suggesting that seagrass meadows, coral reefs and estuaries might be more resilient to long-term changes than we previously thought. Therefore, understanding metacommunity spatiotemporal dynamics is a waypoint to predict the biodiversity changes over ecological and evolutionary time-scales (Chave 2013, García Molinos et al. 2015).

5.3 SMALL-SCALE SPATIAL AND TEMPORAL VARIABILITY ON METACOMMUNITIES: IMPLICATIONS FOR MONITORING BIODIVERSITY

In soft-sediments ecosystems, habitat boundaries are generally difficult to detect (Lohrer et al. 2013, Valanko et al. 2015). Moreover, small-scale spatial (Blanchard 1990, Chapman et al. 2010, Brustolin et al. 2014) and temporal (Morrisey et al. 1992, Tolhurst and Chapman 2005) variability in environmental characteristics and species distributions sometimes can be higher than variations at larger scales (chapters 2, and 3). This might lead to confusions and

misinterpretations in biological responses (Chapman et al. 2010). For example, when we look at the spatiotemporal variation in nematode species richness and sediment properties at estuarine tidal flats (chapter 2), is evident that variation among days and weeks are higher than any monthly or seasonal differences (Fig. 3).

Throughout this thesis I demonstrated that variations at the small spatiotemporal scales were determinant for metacommunity organization and dynamics. Physical, biogeochemical and ecological constraints (e.g., wave exposure, redox profiles, bioengineering) affected species distribution and composition at the scales of meters and hundreds of meters, as well among days and weeks (chapters 2, 3). Although, without temporal replicates at shorter time-scales we wouldn't be capable to properly quantify those variations. In this case, two opposite scenarios are possible: we could either have detected a seasonal pattern (Fig. 3 red dots) or haven't detected it (Fig.3 blue dots) depending on the day sampled. Then the chances to detect differences when they don't really exist (type I error), as well the chances to don't detect differences when they can exist are high (type II error). Similarly, monitoring programs assessing the impacts of anthropogenic disturbances in biodiversity that doesn't have true spatiotemporal replication might reach to wrong conclusions and don't detect any impact of disturbances when they truly exist (Underwood 1997). The occurrence of type II error, as illustrated in the blue dots scenario (Fig. 3) is thus very dangerous and can lead to erroneous conclusions masking potential hazards over ecological communities and human societies.

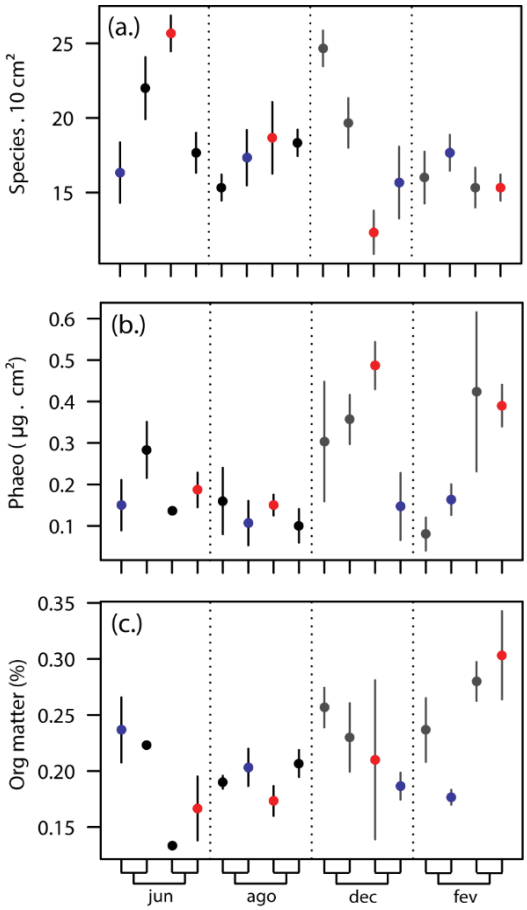

Fig 3. Illustrative hypothetical type I (red dots) and type II (blue dots) errors derived from pseudoreplication of time periods in dynamic subtropical soft-bottom marine ecosystem. The example is based on data about temporal variation in nematode species richness (a.), phaeopigments (b.) and organic matter contents (c.) at estuarine tidal flat sediments (see, chapter 3).

5.4 CONCLUSIONS

Further studies must be made to investigated more profoundly the relationships between metacommunity assembly and landscape characteristics. Manipulative experimental approaches using soft-bottom invertebrate communities as a model can be useful and must be replicated. Although, generalizations of the patterns observed at small-scales to a larger spatial and temporal context should be made with caution. Also, nematode macroecological studies using meta-analysis and/or other statistical approaches must be encouraged, as well data sharing. Metacommunities inhabiting heterogeneous landscapes are shaped by a complex interplay between environmental, spatial, and ecological processes. By assessing

their variation patterns across multiple scales, we can have clues about the processes and mechanisms structuring those metacommunities. Nematode fauna, for example, is structured primary by environmental filtering and niche mechanisms, with coastal habitats harboring distinct communities (e.g., mangroves, tidal flats, and sandy beaches). Variations in community structure within habitats are regulated by distinct mechanisms varying from patch-dynamics at the more protected mangrove habitat to mass-effects in the more exposed sandy beaches. Long-term changes in climate can alter metacommunity organization leading to species extinctions and decreases in beta-diversity. Metacommunity organization will change rapidly over the next years in response to ocean acidification and warming. The development of landscape theories embracing the uniqueness of soft-sediment benthos, as well the increase in data gathering and empirical evidences will bring important advances in metacommunity theory, pushing the discipline boundaries towards an even more holistic interpretation of ecosystems dynamics.

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